

NEMATODE COMMUNITIES AS INDICATORS OF
AGRICULTURAL MANAGEMENT PRACTICES

By

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TABLE OF CONTENTS

	<u>Page</u>
ACKNOWLEDGEMENTS.....	ii
LIST OF TABLES.....	vi
LIST OF FIGURES.....	viii
ABSTRACT.....	x
 CHAPTERS	
1. REVIEW OF LITERATURE.....	1
2. NEMATODE COMMUNITY COMPOSITION UNDER VARIOUS IRRIGATION SCHEMES IN A CITRUS SOIL ECOSYSTEM.....	10
Introduction.....	10
Materials and Methods.....	11
Results.....	14
Discussion.....	16
3. RELATIONSHIP BETWEEN SOIL CHEMICAL STATUS, SOIL NEMATODE COMMUNITY, AND SUSTAINABILITY INDICES.....	26
Introduction.....	26
Materials and Methods.....	28
Results and Discussion.....	32
4. NEMATODE COMMUNITIES AS BIOINDICATORS OF THE STATUS AND PROCESS OF THE SOIL ECOSYSTEM INFLUENCED BY AGRICULTURAL MANAGEMENT PRACTICES.....	42
Introduction.....	42
Materials and Methods.....	44
Results.....	47
Discussion.....	51

5. EFFECT OF VARYING NITROGEN FERTILIZER RATES ON SOIL NEMATODE AND MICROBIAL COMMUNITIES IN A YOUNG CITRUS ORCHARD.....	79
Introduction.....	79
Materials and Methods.....	80
Results.....	83
Discussion.....	85
6. RESPONSE OF SOIL NEMATODE COMMUNITIES TO DIFFERENT GROUND COVERS IN A YOUNG CITRUS ORCHARD.....	99
Introduction.....	99
Materials and Methods.....	101
Results and Discussion.....	103
7. RELATIONSHIP BETWEEN SHANNON-WEAVER DIVERSITY INDEX AND A RECIPROCAL TRANSFORMATION OF THE SIMPSON DOMINANCE INDEX.....	117
Introduction.....	117
Materials and Methods.....	118
Results and Discussion.....	118
8. RESEARCH SUMMARY AND CONCLUSIONS.....	123
REFERENCES.....	127
BIOGRAPHICAL SKETCH.....	134

LIST OF TABLES

<u>Table</u>	<u>Page</u>
2-1 Nematode abundance per 100 cm ³ of soil in samples taken from under the citrus tree canopy.....	20
2-2 Total abundance and trophic group composition per 100 cm ³ of soil in samples taken from under the citrus trees canopy.....	21
2-3 Soil moisture, levels of <i>Phytophthora nicotianae</i> , root biomass, and ecological indices obtained from soil samples taken from under the citrus tree canopy.....	22
2-4 Nematode numbers and ecological indices showing significant ($P \leq 0.05$) differences between treatments receiving smaller (0.43, 0.57, 0.85, and 1.00 ET) amounts of water and treatments receiving greater (1.35 and 1.97 ET) amounts of water.....	23
3-1 Soil chemical measures obtained from soil beneath citrus trees exposed to six different irrigation intensities expressed as a proportions of the evapotranspiration rate (ET) (0.43, 0.57, 0.85, 1.00, 1.32, and 1.97 ET) on 10 February 1996.....	38
3-2 Significant (at $P \leq 0.10$) correlation coefficients between soil chemical measures, nematode genera, and nematode community indices.....	39
3-3 Significant (at $P \leq 0.10$) correlation coefficients between chemical or biological measures and indices of sustainability.....	41
4-1 Analysis of municipal solid waste compost.....	61
4-2 Occurrence of significant effects of water, mulch, and fertilizer treatments, and their interactions on single nematode populations, trophic groups, ecological indices, and other biological and physical characteristics of the soil environment.....	62
5-1 Description of the experimental treatments.....	91
5-2 Analysis of variance results and overall means for nematode genera, trophic groups, and ecological indices.....	92

<u>Table</u>	<u>Page</u>
5-3 Correlation coefficients (r) between fertilizer rate and nematode genus, trophic group, or ecological index.....	94
6-1 Analysis of variance results indicating significance of ground cover treatment effect during three consecutive sampling dates for nematode genus/family, trophic group, and ecological index.....	109
6-2 Means $\pm$ standard deviations for nematode genera/families, trophic groups, and ecological indices.....	111
7-1 Coefficients of regression equations of the Shannon Weaver index (H') on the reciprocal of the Simpson's dominance ($1/\lambda$) or $\ln(1/\lambda)$ for nematode community data sets from four different experiments.....	120

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
4-1 View of treatments separated by the first canonical into two groups: mulch plots and herbicide treatments.....	64
4-2 Abundance of total nematodes (a), bacterivorous nematodes (b), fungivorous nematodes (c), and omnivorous nematodes (d), fungivorous (e) and omnivorous (f) nematodes as a percentage of the total nematode community, and abundance of nematodes associated with decomposition (g) per 100 cm ³ soil in mulch- and non mulch-treated plots.....	65
4-3 Abundance of bacterivorous families or genera: Rhabditiidae (a), <i>Cephalobus</i> (b), <i>Acrobeles</i> (c), <i>Acrobeloides</i> (d), <i>Eucephalobus</i> (e), <i>Teratocephalus</i> (f), <i>Plectus</i> (g), and <i>Wilsonema</i> (h) per 100 cm ³ soil in mulch- and non mulch-treated plots.....	68
4-4 Abundance of fungivorous genera: <i>Aphelenchus</i> (a) and <i>Aphelenchoides</i> (b) per 100 cm ³ soil in mulch- and non mulch-treated plots.....	71
4-5 Abundance of omnivorous genera: <i>Eudorylaimus</i> (a) and <i>Aporcelaimellus</i> (b) per 100 cm ³ soil in mulch- and non mulch-treated plots.....	72
4-6 Abundance of herbivores: <i>Belonolaimus</i> (a), <i>Criconemoides</i> (b), and root associates (Tylenchidae) (c) per 100 cm ³ soil in mulch- and non mulch-treated plots.....	73
4-7 Indices of diversity: richness (a), Shannon-Weaver trophic diversity (b), Shannon-Weaver genus diversity (c), Simpson genus diversity (d), and Simpson genus dominance in mulch- and non mulch-treated plots.....	74
4-8 Bacterivores + fungivores to herbivores ratio (a) and fungivores to bacterivores ratio (b) in mulch- and non mulch-treated plots.....	76
4-9 Maturity indices: plant-parasitic index (a), maturity index (b), and total maturity index (c) in mulch- and non mulch-treated plots.....	77

<u>Figure</u>	<u>Page</u>
4-10 Soil moisture (a), <i>Phytophthora</i> density (b), and primary production (fruit yield) (c) in mulch- and non mulch-treated plots.....	78
5-1 Abundance of bacterial feeding nematodes: Rhabditidae (a) and <i>Eucephalobus</i> (b) at four different nitrogen rates.....	95
5-2 Abundance of <i>Acrobelus</i> at four different nitrogen rates.....	96
5-3 Abundance of <i>Aphelenchus</i> at four different nitrogen rates.....	96
5-4 Abundance of <i>Belonolaimus</i> at four different nitrogen rates.....	96
5-5 Abundance (a) and percentage of the total nematode community (b) of omnivorous nematodes at four different nitrogen rates.....	97
5-6 Maturity index values in four different nitrogen rates.....	97
5-7 Densities of bacterial (a) and fungal (b) colony forming units at four different nitrogen rates.....	98
6-1 Abundance of <i>Acrobeloides</i> (a), <i>Aphelenchus</i> (b), <i>Eudoryalimus</i> (c), and miscellaneous plant parasites (d) per 100 cm ³ under four ground cover types.....	113
6-2 Abundance of bacterivores (a), fungivorous nematodes (b), and percentage of omnivorous nematodes (c) per 100 cm ³ under four ground cover types.....	115
6-3 Richness (a) and total maturity index (b) under four ground cover types.....	116
7-1 Linear (a) and logarithmic (b) regression models relating Shannon-Weaver index to reciprocal of Simpson index for soil nematode community data in a factorial experiment.....	121
7-2 Linear (a) and logarithmic (b) regression models relating Shannon-Weaver index to reciprocal of Simpson index for overall soil nematode community data pooled over four experiments.....	122

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Interest in the sustainability of farming practices has increased in response to environmental problems associated with conventional agricultural management. Availability of measures of the status of the soil ecosystem is important for environmental assessment and monitoring programs, as well as for farm managers who could modify their farming practices. This study investigated the effects of various agricultural management regimes comparing standard vs. reduced-input practices (irrigation rates, fertilization rates, and types of ground cover) on the community structure of the nematode fauna in a citrus orchard in the sandy ridge area of Central Florida. Ecological measures such as community structure indices, diversity indices, and maturity indices were assessed and related to different agricultural regimes.

From the various indices of community structure, maturity indices were most effective in distinguishing differences among agricultural regimes. In an irrigation experiment (six rates), maturity indices indicated history of water usage. In a multifactorial experiment (fertilization, irrigation, and type of ground cover; each factor at two levels), maturity indices, unlike diversity

indices, indicated the status and intensity of soil processes (decomposition, mineralization). Maturity indices were also the only measures at the index level responding consistently and significantly to fertilizer rates. Typically, decrease of maturity indices resulted from stronger pulses of “disturbance” (low irrigation rates, high fertilization rates, mulch additions, bare soil). Overall, maturity indices provided sufficient information to be efficient tools for quick estimation of soil condition.

Trophic groups might be useful in describing general and overall trends of the soil ecosystem status; however, use of trophic groups may sometimes obscure information. Trophic grouping of bacterial feeders (*r*- and *K*-strategists) averaged different (often of opposite nature) nematode responses observed at the generic level. In contrast, grouping of omnivorous nematodes (*K*-strategists only) reinforced often weak patterns observed at the generic level.

The effects of farming practices on microflora, root biomass, the status of macro- and micronutrients in the soil, and on sustainability measures (productivity, resource use efficiency, and profitability) were also examined, and were found to be related to nematode components of the soil citrus ecosystem.

CHAPTER 1 REVIEW OF LITERATURE

Decline of quality of natural and managed ecosystems has concerned a variety of people from many disciplines, ranging from farmers to scientists. The quality of agricultural systems is a particularly sensitive subject for political, environmental, and socio-economic discussions. The ongoing conflict between production of sufficient amounts of food for an ever-growing human population and preservation of natural resources stimulates the search for alternative ways of food and fiber production.

Agricultural management practices affect soil characteristics. Reduction in diversity of soil biota (Paoletti et al., 1992), loss of soil organic matter content, and increased soil erosion (Ehrlich, 1988) are problems in many conventional agricultural systems. Other effects from farming systems that use high amounts of matter and energy (fertilizers, pesticides, irrigation, and cultivation) are ground- and surface water contamination, posing potential hazards to human and animal health (Schaller, 1993). In recent years, because of environmental and social concerns, sustainable agriculture has become a new paradigm for the agriculture (Schaller, 1993; Kenneth et al., 1996). Although many people use the term, an understanding of “sustainable agriculture” may vary from one person to another. Most people, however, place an emphasis on preservation of natural resources through reduced input of synthetic materials and energy, and on management practices that favor higher dependence on natural biological ecosystem processes done in a way to economically sustain food production (Elliot and Cole, 1989). Successful transition from current to more sustainable agroecosystems will require detailed information on

all components of the ecosystem as well as appropriate measures to evaluate environmental quality.

Soil is a complex and dynamic ecological system (Edwards and Stinner, 1988), made up of mineral particles, organic materials, and interstitial spaces filled with air and water. Organic materials in the soil consist of plant litter, dead and living bodies of soil inhabitants, and the chemical products of their activities (Lee and Pankhurst, 1992). Soil ecosystems influence functioning of the above-ground ecosystems through nutrient (e.g., C and N) and energy cycles and fluxes. To detect changes of the soil condition (e.g. nutrient enrichment, loss of soil fertility, accumulation of heavy metals, and loss of diversity), various indicators may be monitored. The soil biota is an excellent prospect for biomonitoring, because it integrates physical and chemical soil properties over a certain period of time.

Nematodes possess several attributes of any prospective bioindicator (Cairnes et al., 1993). They are abundant in virtually all environments ranging from dry deserts to benthic sediments to polar ice caps (Niblack, 1989). They represent diversity of life strategies (colonizers vs. persisters) and feeding habits (algal, bacterial, and fungal feeders, herbivores, omnivores, predators, and root associates) (Yeates et al., 1993a). A hand full of soil contains tens to hundreds of nematode species (Ettema, 1998). Their relatively short life cycles should allow for quick responses to changes in the soil environment. In addition, information on sampling, extraction, and preservation methods is more defined for nematodes than for other soil organisms (Freckman, 1988).

Analysis of nematode community structure has been successfully used to illustrate the effects of heavy metal pollution and acidification (Bongers et al., 1991; Ruess et al., 1996). Many studies have examined nematode communities with respect to natural ecosystem succession and environmental disturbance (Ettema and Bongers, 1993; Freckman and Ettema, 1993; de Goede et al., 1993; Wasilewska 1994; Yeates and Bird, 1994). The effects of selected

management practices on the entire nematode community in agroecosystems have been also investigated (Ferris et al., 1996; Freckman and Ettema, 1993; McSorley and Frederick, 1996; Todd, 1996; Yeates et al., 1993b).

Interpretation of changes in nematode community structure, influenced by seasonal or anthropogenic factors, can be based on a direct analysis of the dynamics of all identified taxa, or on data derived from taxonomic or ecological indices. Some of the measures for comparisons of nematode communities include genus, family, and total nematode densities, trophic composition, and diversity measures. Because identification of nematodes to species or generic level is somewhat troublesome, family or functional group level is often preferred. The ability to predict functions and changes of ecosystems, however, likely requires understanding of nematode relationships in more detail than at the family level of resolution. Although characterization of trophic interactions might be valuable for general inferences about global flows of energy and matter, misinterpretation of biotic interactions at the scale of a single agricultural field is highly possible. Different responses, often of opposite nature, of individual species can be neutralized by grouping within the same functional group, therefore inadequately illustrating the status and/or ecosystem processes.

An alternative way of looking at nematode community patterns is by use of ecological indices. A variety of indices are available for the measurement of diversity at the community level (Ludwig and Reynolds, 1988). Richness measures the total number of species. Two other indices that integrate information about the number of species and their relative abundances are Shannon-Weaver diversity index (Shannon and Weaver, 1949)

$$H = - \sum p_i \log p_i$$

where p_i is the proportion of the i th species in the sample, and Simpson dominance index (Simpson, 1949)

$$\lambda_i = \sum (p_j)^2$$

An alternative measure of diversity is a reciprocal transformation of the Simpson's dominance index

$$1/\lambda_i = 1/\sum(p_j)^2$$

In studies of soil nematode communities, the generic or even trophic level is often substituted for the species level in computing diversity, due to difficulty in recognizing undescribed species (Wasilewska, 1979; Freckman and Ettema, 1993). The lower the level of identification resolution, however, the less informative and adequate the index will be. One of the major weaknesses of diversity indices is that they are devoid of biological information and reflect purely quantitative characteristics of the community. Ecological requirements of the constituent species and their life strategies are irrelevant and not considered in the derivation of those metrics.

Some insights on the decomposition and nutrient mineralization pathways and primary production can be obtained by calculating a ratio of fungivorous to bacterivorous nematodes (F/B), and a ratio of detritivores to plant parasites ($F+B/PP$), respectively (Freckman and Ettema, 1993; Wasilewska, 1994). Although these indices improve understanding of soil processes, they disregard information about the complexity and quality of food webs.

Two more biologically meaningful indices were proposed specifically for nematode communities by Bongers in 1990. Maturity index (MI) and plant parasitic index (PPI) are semiquantitative measures. Nematode families are ranked (1-5 *c-p* value) on a scale from 1 for colonizers (*r*-strategists) to 5 for persisters (*K*-strategists). In general, colonizers are characterized by very short life cycles (few days), high reproductive and colonization ability, population density fluctuations, and relatively high tolerance to disturbance and pollution (Bongers, 1990). They usually dominate nematode communities, particularly in unpredictable and stressed environments. Persisters, on the other hand, have long life cycles (up to several years) and low reproduction and colonization ability. Their densities do not fluctuate greatly

over time, and tolerance to disturbance is very limited. They are never dominant, but comprise a consistently higher proportion of the nematode community in stable and unstressed habitats.

Except for omnivorous and predacious nematodes (*c-p* 4-5), all trophic groups can be represented by both colonizers and persisters. The *MI* is calculated as a weighted mean of the individual *c-p* values:

$$MI = \sum v_i * f_i$$

where v_i is the *c-p* value of *i*th family and f_i is the frequency of that family in a nematode community. While *MI* relates to “free-living” component of nematode community (algal, bacterial, and fungal feeders, omnivores, and predators, but not plant parasites), the *PPI* contains information exclusively on plant-parasitic families. The formula is the same as that for *MI* except that total abundance of plant-parasitic nematodes is the basis for calculation of the frequencies of plant-parasitic families. To avoid separation of trophic habits, a modified maturity index or total *MI* ($\sum MI$) was proposed by Yeates (1994). $\sum MI$ includes both free-living and plant-parasitic nematodes. Nematode communities dominated by colonizers in stressed or unstable habitats would be expressed by low values of maturity indices, while those in unstressed or stable environments characterized by higher proportions of persisters would be described by relatively higher values.

The ability to detect different patterns in nematode communities along an environmental gradient depends on the choice of the right measures. This information is particularly important for environmental monitoring and assessment programs. However, the ability to monitor and assess the quality of soils would be also of importance to farm managers, who could modify their farming strategies and thus prevent ecological degradation of agroecosystems and the surrounding environment.

In recent years, several researchers investigated whether, and to what extent, different agricultural practices can be illustrated by measures of nematode community structure. In

general, different nematode measures reflected differences between undisturbed and human-impacted environments (Freckman and Ettema, 1993; Wasilewska, 1994; Yeates and Bird, 1994). Burning and N fertilization stimulated bacterial feeding nematodes, but caused declines in omnivorous and predacious nematodes (Todd, 1996). Fungal feeders were indicative of the transition from conventional to organic grassland production systems (Yeates et al., 1997). Diversity and maturity indices were usually lower in agricultural fields and greater in successional or native ecosystems. Within agricultural fields, nematode maturity index values were shown to be greater in rows than between rows of a given crop (McSorley and Frederick, 1996), and under perennial than annual crops (Neher and Campbell, 1994). In organic farming systems, abundances of bacterial feeding *r*-strategists were higher than in conventional agricultural systems, resulting in lower maturity index values (Ferris et al., 1996). Nematodes have also been proposed as useful in defining sustainable agroecosystems (Neher and Campbell, 1993).

Typically, to evaluate the quality of the agroecosystem or its state of sustainability, results obtained from agriculturally managed sites would be compared to native, undisturbed sites, or to sites under long-term secondary succession. This procedure might create significant problems. The very nature of any ecosystem, characterized by a nearly infinite number of variables, implies that sustainability can be defined in terms of many possible variables or combinations of variables. These variables include items such as biodiversity, levels of specific mineral elements and pollutants, or levels of primary production. Components of sustainability of agroecosystems and natural systems may differ (e. g., soil disturbance, vegetation diversity, and nutrient loss). To assess the degree of sustainability of an agroecosystem, it should be compared to known healthy system (e.g., productive, profitable, and environmentally compatible) or, in contrast, to a degraded and unhealthy agroecosystem unproductive,

unprofitable, and environmentally risky), rather than to native ecosystems. In this way, the comparison becomes more adequate and appropriate.

In agroecosystems, profitability is often the most important variable from the farmer's point of view. Moreover, the most profitable practices will be the most sustainable ones for a given set of economic conditions because of market competition. Profitability estimates could include broad aspects of the agroecosystem (e.g. hidden management costs such as cost of environmental remediation, projected future values of limited resources, and estimated value of biodiversity), and thereby provide a reasonable index of sustainability by revealing the optimum relationship between crop production and management inputs (Duncan and Noling, 1998).

Florida citrus orchards of the central ridge are ideal for testing hypotheses concerning usefulness of nematode communities as bioindicators of ecosystem status and processes for several reasons. These perennial systems provide relatively stabilized soil environments, reducing variation from physical and chemical soil disturbances typical of annual agricultural systems (tillage, crop type, density of decaying roots). The soil of central ridge of Florida (planted mostly to citrus) is exclusively sandy (up to 96%) with low organic matter (1%) content, reducing the variation associated with different experimental sites (soil types and climatic conditions). The climate is subtropical with an annual mean temperature of 22°C and annual mean precipitation of 109 cm. There is little information on nematode community structure in citrus agroecosystems and on the disturbance to nematode communities produced by agricultural practices in citrus orchards. If we could show that nematode communities respond to various gradients of an agricultural practice, we could provide necessary evidence that nematodes indeed are useful for biomonitoring and quantifying the status and sustainability of an ecosystem.

I hypothesized that as material and energy inputs in a citrus orchard agroecosystem decrease from excessive to more conservative to insufficient levels, the nematode community structure would change with values of the input variable. Moreover, this strategy would allow us

to determine energy usage (inputs and outputs) and costs for various treatments, and thus relate nematode community measures gradients to some measures of ecosystem sustainability (energy usage, profitability, resource conservation).

In this study, I focused on irrigation, fertilization, and ground cover. It is unknown whether these different agricultural practices affect nematode community structure in a predictable way. Typical management practices involve growing citrus trees in vegetation-free rows (to reduce competition for nutrients and water between trees and weeds) with grassy areas between rows of trees (to minimize soil erosion). The sandy soils, on which most Florida citrus is grown, are highly vulnerable to nutrient leaching. High sand content and a small fraction of organic matter result in low soil fertility, low cation exchange capacity, and low moisture-holding capacity (Jackson et al., 1995). Successful citrus production in Central Florida requires considerable fertilizer and water inputs. While higher nitrogen additions usually improve crop yields (unless the amounts are excessive) (Koo, 1988), they also increase the risk of groundwater pollution with nitrates. Nutrient loss through the soil profile is magnified by the leaching power of rainfall and irrigation. To reduce risk of nitrates in groundwater and conserve water resources, standard agricultural practices need to be modified. Many citrus growers already implement N and water conservation techniques (e.g., additions of smaller but more frequent nitrogen rates, more precise placement, more efficient irrigation management). In addition, growing cover crops in citrus orchards has been considered as an alternative management to weed-free (herbicide treated) citrus tree rows. A potentially good candidate cover crop is perennial leguminous peanut (*Arachis glabrata*). The benefits of perennial peanut to citrus production could be multiple: 1) contribution of nitrogen to the system through N_2 -fixation; 2) lowering soil temperatures through shading; 3) improvement of soil moisture conditions; 4) reduction of synthetic fertilizer rates; and 5) reduction of groundwater pollution.

Manipulation of the above-mentioned agricultural practices, to simulate sustainable vs. unsustainable systems, would allow us to monitor nematode communities in soil under conditions of different management practices, but of the same physico-chemical soil properties, vegetation cover, and climatic conditions. Therefore, the overall objectives of this study were to 1) characterize nematode communities (taxonomic and ecological index description) in different agricultural regimes; 2) evaluate whether different management practices affect soil ecosystems and thus nematode communities to a similar extent; 3) determine whether soil differences can be reflected by nematode characterization (key taxa or indices); 4) determine which measures of ecological characterization are the most useful in differentiating various agricultural regimes; and 5) determine which of the ecological measures most adequately illustrate the status and processes of the soil environment.

CHAPTER 2

NEMATODE COMMUNITY COMPOSITION UNDER VARIOUS IRRIGATION SCHEMES IN A CITRUS SOIL ECOSYSTEM

Introduction

Conventional agricultural systems have been identified as a significant source of environmental degradation (excessive consumption of nonrenewable resources, ground water pollution, lake eutrophication, soil erosion, and loss of biodiversity) (Ehrlich, 1988). In recent years, because of environmental and social concerns, more interest has been devoted to developing sustainable systems. Emphasis is placed on conservation of natural resources through reduced inputs of synthetic materials and energy, and management practices that favor dependence on natural biological ecosystem processes (Elliot and Cole, 1989). Successful transition from current to more sustainable agroecosystems, however, will require detailed information on all components of the ecosystem and appropriate biological measures to evaluate environmental quality.

Nematodes may be useful bioindicators for estimating environmental quality and disturbance because they are numerous in all environments, where they exhibit diverse life strategies and feeding habits (Freckman, 1988; Yeates et al., 1993). Bongers et al. (1991) related analysis of the nematode community to heavy metal pollution and acidification. Many studies have examined nematode community structure with respect to natural ecosystem succession and environmental disturbance (Ettema and Bongers, 1993; Freckman and Ettema, 1993; de Goede et al., 1993; Wasilewska, 1994; Yeates and Bird, 1994). The effects of management practices on the entire nematode community in agroecosystems also have been investigated (Ferris et al., 1996; Freckman and Ettema, 1993; McSorley and Frederick, 1996; Yeates and Bird, 1994).

Interpretation of changes in community structure can be based on a direct analysis of the dynamics of all taxa, or on ecological indices derived from taxonomic data. Environmental disturbance, including agricultural management practices, could be revealed with appropriate diversity and maturity indices. Diversity and maturity indices values were usually less in agricultural fields and greatest in successional or native ecosystems. Within agricultural fields, nematode maturity index values (Bongers, 1990) were shown to be greater in rows than between rows of a given crop (McSorley and Frederick, 1996), and lower in organic farming systems, which resulted from an increased density of bacterivorous *r*-strategists (Ferris et al., 1996).

There is no information on nematode community structure in citrus agroecosystems and on the disturbance to nematode communities produced by agricultural practices in citrus orchards. We hypothesized that as material and energy inputs (fertilizer, water, herbicide) in a citrus agroecosystem were decreased from excessive to more conservative or insufficient levels, nematode community structure would change with values of the input variable. The objectives of this study were to compare nematode communities over a gradient of irrigation in a citrus orchard, and to determine which nematode community measures were effective in differentiating irrigation treatments.

Materials and Methods

The experiment was conducted at the University of Florida Citrus Research and Education Center (CREC) in Lake Alfred, Florida. The soil type was Astatula fine sand with pH 6.2 and 0.7 % organic matter. The experimental site had been planted to citrus trees (*Citrus* spp.) for the last 50 years. Young citrus trees of 'Hamlin' orange (*Citrus sinensis* (L.) Osbeck) on 'Swingle' citrumelo (*Poncirus trifoliata* x *Citrus paradisi*) rootstock free of endoparasitic nematode pests were planted on 10 April 1991. The experiment was initiated 1 June 1991 and nematode data collection began in 1995. The trees were spaced in rows 6 meters apart, with 3.7 meters between trees in rows. During 1991-1996, nutrient management followed the fertilizer

guidelines typical for citrus in Florida (Ferguson et al., 1995). To control weed growth under the tree canopies, plots received glyphosate (N-(phosphonomethyl) glycine, isopropylamine salt) herbicide treatment as needed. Generally, glyphosate was applied 3-4 times per year at approximately 2-month intervals starting in late February.

Trees were watered every fourth day at 6 different irrigation intensities starting 1 June 1991. Irrigation intensities were expressed as a proportion of the evapotranspiration rate (ET) (0.43 ET, 0.57 ET, 0.85 ET, 1.00 ET, 1.32 ET, and 1.95 ET). These proportions were achieved by using different size openings of microsprinkler emitters in the irrigation system. To calculate the amount of water required for each month, data from the previous years (app.70 years) of ET measurements were used. For example, if ET for January of previous years was 0.18 cm of water per day, the treatment 1.00 ET would receive 0.72 (0.18 cm x 4 days) cm of water every fourth day in January. Other treatments would receive an assigned proportion of that amount; for instance 0.43 ET would receive $0.72 \times 0.43 = 0.31$ cm of water every fourth day. Monthly ET typically varied from a low of 0.18 cm for January to a high of 0.46 cm of water in May.

The experiment was a randomized complete block design with each treatment replicated four times. Data collection started four years after planting which allowed nematode community structures sufficient time to reach an equilibrium. Soil samples consisting of 16 soil cores (2-cm diameter and 30-cm length) taken from under the tree canopy in a diagonal transect pattern were collected on 30 May 1995, 09 September 1995, 10 February 1996, and 16 May 1996. The soil cores were mixed and passed through a sieve (2 mm by 2 mm mesh size) to separate root tissue from the soil. A soil subsample of approximately 100-cm³ was used immediately to estimate populations of *Phytophthora nicotianae* Breda de Haan (Timmer et al., 1988), which is a pathogen causing citrus root decay. The remaining soil was stored in sealed plastic bags at 10 °C for up to 15 hours. Nematodes were then extracted from 100-cm³ subsamples by wet sieving followed by centrifugation (Jenkins, 1964). Extracted nematodes were killed with heat, counted,

and identified primarily to genus with the aid of an inverted microscope. To determine soil moisture, 4 to 7 g of soil from each soil sample was oven-dried for about 24 h at 60 °C.

The abundance of each nematode genus expressed as numbers of specimens of the same genus, the total number of nematodes of different genera, and the total number of genera (richness) in each sample were recorded. Nematode genera were assigned to the following trophic groups: algivores, bacterivores, fungivores, omnivores, plant associates, plant parasites, or predators (Yeates et al., 1993), and decomposition associates (bacterivores and fungivores together). The total abundance of nematodes in each trophic group and the percentage of each trophic group within the entire nematode community were determined for each sample.

Based on the genera and trophic group densities, several ecological indices of nematode community structure were calculated. The Shannon-Weaver diversity index (1949) was used to compare diversity of either genera or trophic groups:

$$\text{genus (g) or trophic group (t) diversity} = H_g \text{ or } H_t = - \sum p_i \log_e p_i \quad (1)$$

where p_i is the proportion of the i th genus or trophic group in the sample. Simpson's (1949) index

$$\text{genus (g) or trophic group (t) dominance} = \lambda_g \text{ or } \lambda_t = \sum (p_i)^2 \quad (2)$$

was used to compare either generic or trophic group dominance. A reciprocal transformation of the Simpson's dominance index offers an additional measure of diversity either at genus or trophic group level (Freckman and Ettema, 1993):

$$\text{Simpson's genus (g) diversity} = 1/\lambda_g \quad (3)$$

Maturity indices MI and PPI were calculated following Bongers (1990). The MI is a weighted mean of the individual $c-p$ values:

$$MI = \sum v_i \cdot f_i \quad (4)$$

where v_i is the $c-p$ value of taxon i and f_i is the frequency of that taxon in a sample. $C-p$ values, ranging from 1 for colonizers (c) to 5 for persisters (p), are assigned to nematode families to

illustrate their life strategies and, thus, conditions of the surrounding environment (Bongers, 1990). While *MI* relates to the “free-living” component of nematode community (bacterial, fungal, and algal feeders, omnivores, predators, and plant associates, but not plant parasites), the plant-parasitic index (*PPI*) contains information exclusively on plant-parasitic families. The formula is as for *MI* except that total abundance of plant-parasitic nematodes is the only basis for calculation of frequencies. To avoid separation of trophic habits, a modified maturity index or total *MI* (ΣMI) (Yeates, 1994) was also calculated. ΣMI includes both plant-parasitic and free-living nematodes. Fungivore- to bacterivore- ratio (F/B) (Freckman and Ettema, 1993) and the ratio of fungivores + bacterivores to plant parasites (F+B/PP) (Wasilewska, 1994) were determined to illustrate decomposition and nutrient mineralization pathways and primary production. Estimation of primary production was accomplished by harvesting trees in February 1995 and 1996. However, since the trees had not yet reached their productive maturity, we pooled the two harvesting seasons into one crop yield value to better represent the tree fruit production.

Effects of irrigation intensity on the above measures of nematode community structure were determined by analysis of variance, single degree of freedom orthogonal contrasts, and correlation analysis with SAS software (SAS Institute, Cary, NC)

Results

The abundance of the most numerous nematode genera are listed in Table 2-1. The densities of the following rare or occasional genera were not listed in the table, but were included in their trophic groups: *Alaimus*, *Bunonema*, *Chronogaster*, *Teratocephalus* (bacterivores); *Diphtherophora* (fungivore); *Mononchus*, *Mylonchulus*, *Prionchulus*, *Carcharolaimus*, *Nygolaimus* (predators); *Mesodorylaimus* (omnivore); *Monochromadora* (algivore). Other Dorylaimida were *Ecumenicus* and *Pungentus* spp., and miscellaneous plant parasites included *Meloidogyne*, *Tylenchulus*, *Trichodorus*, and *Xiphinema* spp. The abundances of most nematode

genera did not appear to respond to the irrigation gradient in any predictable manner. However, the abundance of *Aporcelaimellus* and *Eudorylaimus* spp. had patterns with season and irrigation treatments throughout the year. Irrigation rate was correlated significantly ($P \leq 0.05$) with numbers of *Eudorylaimus* spp. on all sampling occasions ($r = 0.6$ for May 1995, $r = 0.5$ for October 1995, $r = 0.4$ for February and May 1996) and for *Aporcelaimellus* spp. only in May ($r = 0.5$) and October ($r = 0.7$) of 1995. According to orthogonal contrasts, the genus *Eudorylaimus* was effective only in distinguishing treatments with greater (1.95, 1.32 ET) versus lower (1.0, 0.85, 0.57, 0.43 ET) irrigation levels in May and October 1995, and February 1996 ($P \leq 0.05$), and the genus *Aporcelaimellus* was effective only in May and October of 1995 ($P \leq 0.05$) (Table 4). Some of the bacterivorous genera such as *Acrobeles*, *Eucephalobus*, *Prismatolaimus*, and *Zeldia* seemed to show seasonal patterns with higher densities in May as compared to February 1996 or October 1995, but they did not show a significant response to irrigation intensity.

Total abundance of nematodes per 100-cm³ soil was relatively low and ranged from 220 in October 1995 to 572 in May 1995 (Table 2-2). Total densities were more influenced by the time of sampling than by irrigation rate. Typically, higher numbers of total nematodes were found in May of both years. Of the trophic groups, only omnivores were affected ($P \leq 0.05$) by the irrigation intensity (Table 2-4). A positive correlation of omnivore numbers with irrigation intensity was persistent throughout the entire year of sampling and ranged from $r = 0.4$ in February 1995 to $r = 0.59$ in May 1995. Correlation coefficients were improved when omnivores were expressed as a proportion of the entire nematode community. While the omnivores abundance measure was useful in differentiating treatments with greater vs. smaller water applications during May and October of 1995, and February of 1996, omnivores expressed as the percentage of the nematode community differentiated treatments on all sampling dates ($P \leq 0.05$) (Table 2-4).

Nematode communities were dominated by bacterial feeders, which comprised 54 to 73% of the total nematode community. Fungivores and omnivores were the next most abundant trophic groups. Root associates (i.e. Tylenchidae) and plant parasites were similarly abundant and their proportions ranged from 2 to 11%. Predatory and algivorous nematodes were least common and were entirely absent in many samples (Table 2-2).

Generally, generic richness was greatest in May and lowest during February 1995 (Table 2-3). No response to irrigation intensity could be observed. Shannon-Weaver diversity indices for genus and trophic groups did not vary significantly with either season (Table 2-3) or irrigation rate. Genus and trophic group dominance were more variable, but no consistent tendency was present. Simpson's diversity responded only to seasonal change, and was lowest in February 1996 (5.55 - 7.00) and highest in May (7.14 - 9.20). Of all the indices of nematode community structure, only maturity indices were affected by the irrigation treatments. Positive correlations ($P \leq 0.05$) with irrigation intensity were observed on all sampling dates for MI ($r = 0.58, 0.48, 0.39, \text{ and } 0.69$) and for ΣMI ($r = 0.48, 0.52, 0.47, \text{ and } 0.66$). Both indices could distinguish treatments receiving greater amounts of water (1.95 and 1.32 ET) (contrast procedure) from treatments with smaller irrigation levels ($P \leq 0.05$) (Table 2-4). The ratios F/B and F+B/PP varied considerably with respect to season and irrigation rate, but without any consistent pattern (Table 2-4).

Irrigation treatments were not reflected in either soil moisture or root biomass (Table 2-3). *Phytophthora nicotianae* levels tended to increase as the experiment progressed from May 1995 to May 1996, but were not correlated with irrigation intensity. In general, an increasing water rate positively affected fruit production. However, increases higher than 0.85 ET did not result in statistically significant ($P \leq 0.05$) yield improvements.

Discussion

Irrigation is an important management practice in many citrus orchards world wide, as well as in Florida. Higher water application rates usually lead to higher tree growth and yield of fruit. Excessive use, however, may not result in additional yield increase. The wide range of irrigation in this experiment represented various water supply schemes used by citrus growers. In this study, nematode community structure was clearly influenced by the water treatments. Of the many nematode genera representing all trophic groups, only omnivores (*Aporcelaimellus* and *Eudorylaimus* spp. in particular) responded strongly to irrigation levels. Their total numbers and relative proportions increased steadily with increased irrigation. The lack of correlation between omnivores and soil moisture suggests that an increase of omnivores can be explained by long-term effects of elevated water supply rather than by the temporary soil water content. The very high sand content in Florida soils exposed to rather high temperatures results in frequent soil water fluctuations through drying. Continuous applications of higher water rates probably stabilize the soil environment and create better opportunities for establishment of nematode communities with a higher proportion of *K*-strategists which seems to replace some of the bacterial feeders. Of the various trophic groups, only omnivores, particularly when expressed as the proportion of the nematode community, were useful in detecting smaller and greater irrigation regimes on almost all sampling occasions. An increase of omnivorous nematodes in irrigated plots also was observed in a Swedish pine forest (Sohlenius and Wasilewska, 1984). Other *K*-strategists, such as predacious nematodes, were very rare in our soil samples, which is rather typical for agroecosystems (Ferris et al., 1996; Freckman and Ettema, 1993; McSorley and Frederick, 1996; Wasilewska, 1994; Yeates and Bird, 1994).

Although bacterivores and plant parasites did not respond to water treatments in this citrus orchard, their abundances differed from other agroecosystems. Bacterial feeders were very abundant in the study soil, comprising 65-72% of the total nematode community compared

to about 50% for Florida soybean fields and Polish meadows (McSorley and Frederick, 1996; Wasilewska, 1994), and 20-30% for some south Australian soils (Yeates and Bird, 1994). This predominance of bacterivores may be associated with their particular adaptations to highly variable soil environments with very low soil organic matter content. Citrus root decay caused by *Phytophthora nicotianae* may also influence the abundance of bacterivores. However, as a result of relatively low *P. nicotianae* population numbers, increased root decay and was not observed in our study. The densities of plant-parasitic nematodes were unusually low (2-10%), especially when compared to studies mentioned above that reported them comprising 30-50% of the total community. Such a low proportion of plant parasites probably reflects the use of a citrus nematode resistant rootstock free of other endoparasitic nematode pests, and to cover management practices under the trees. To minimize the competition for water and nutrients between the citrus tree and other plants, the ground directly under the tree is kept free of any vegetation (treated with herbicide). Exclusion of additional roots that could provide food resources for many plant-parasitic nematodes most probably limited their abundance and diversity. Also, citrus has few endoparasites, but few were present in this grove. *Belonolaimus* spp. were consistently present in all soil samples since it feeds on citrus roots (Duncan and Cohn, 1990) as well as weed hosts. Periodic weed outgrowths allowed the maintenance of populations of other plant parasites (*Criconemoides* and *Hoplolaimus* spp.), but at much lower numbers. Sporadic appearance of *Meloidogyne*, *Trichodorus*, and *Xiphinema* spp. suggested the weeds were their preferred hosts.

Some of ecological indices have been used effectively for comparisons of ecosystems. In the present study, maturity indices were the only measures sensitive to irrigation. Maturity index and the total maturity index were equally effective in separating low vs. high irrigation management schemes. Higher values of maturity indices with increased irrigation were caused by the higher proportions of omnivorous, suggesting that communities are more stable under

high irrigation regimes. Maturity indices or omnivorous nematodes could illustrate the water management history. For example higher values of *MI* or higher proportions of omnivores above a specified level would indicate excessive use of water. As such maturity index and omnivorous nematodes may be useful indicators of irrigation history in citrus orchards. Whether similar results could be obtained under different experimental conditions (e.g. fertilization rates, fertilizer type, pest management, and rainfall) is an important question that requires further investigation. The plant-parasitic index was not useful since predominant parasites were in the $c-p = 3$ group. Subtle deviations from $PPI = 3$ simply illustrated occasional appearances of *Xiphinema* and *Trichodorus* spp.

From the point of view of nematode community structure, very well irrigated citrus soils in Florida are more stable since they promote conditions under which the nematode species most sensitive to environmental stress (omnivorous *K*-strategists) can exist. Long-lived perennial citrus roots, with limited invasion by short-lived weed hosts (herbicidal treatments) that promote *r*-strategists, would support this condition. It has been demonstrated that strong fluctuations of soil moisture in the upper 15 cm negatively affects root life span, thus, soil that is irrigated well on a regular basis would promote more stable root system (Eissenstat and Yanai, 1997). However, conservation of natural resources, including water, is a major goal of sustainable agriculture. What is sustainable for some nematodes may not necessarily be sustainable from the agroecosystem management perspective. Indeed, our yield data indicated that irrigation rates above 0.85 ET were wasteful of water and energy. In this scenario, sustainable water management practices for maximum yield efficiency (i. e. fruit/unit H₂O) probably do not coincide with the stability or sustainability of the soil citrus nematode communities.

Table 2-1. Nematode abundance per 100 cm³ of soil in samples taken from under the citrus tree canopy.

Feeding habit	Taxonomic unit	c-p value ^a	May 1995	October 1995	February 1996	May 1996
Bacterivores	Monhysterida	1	4 ± 4 ^b	2 ± 2	1 ± 2	2 ± 2
	Rhabditida	1	40 ± 29	24 ± 14	27 ± 15	21 ± 9
	<i>Acrobes</i>	2	73 ± 37	48 ± 26	71 ± 44	59 ± 21
	<i>Acrobeloides</i>	2	21 ± 17	3 ± 6	3 ± 4	20 ± 13
	<i>Cephalobus</i>	2	62 ± 30	48 ± 21	78 ± 37	72 ± 28
	<i>Eucephalobus</i>	2	13 ± 7	7 ± 6	8 ± 7	9 ± 7
	<i>Plectus</i>	2	1 ± 2	2 ± 2	1 ± 1	2 ± 3
	<i>Wilsonema</i>	2	5 ± 5	8 ± 7	7 ± 7	3 ± 3
	<i>Zeldia</i>	2	58 ± 30	14 ± 9	30 ± 17	51 ± 24
	<i>Prismatolaimus</i>	3	18 ± 13	8 ± 11	5 ± 6	9 ± 10
Fungivores	<i>Aphelenchoides</i>	2	4 ± 3	2 ± 2	4 ± 5	1 ± 3
	<i>Aphelenchus</i>	2	79 ± 38	36 ± 15	75 ± 36	56 ± 23
Predators	<i>Prionchulus</i>	4	4 ± 5	0 ± 1	0 ± 0	0 ± 1
Omnivores	Dorylaimida	5	6 ± 7	10 ± 7	3 ± 3	9 ± 7
Root associates	Tylenchida	2	32 ± 44	15 ± 15	13 ± 12	15 ± 12
Plant parasites	<i>Belonolaimus</i>	3	4 ± 7	1 ± 1	2 ± 2	3 ± 4
	<i>Cricanemoides</i>	3	2 ± 4	1 ± 2	2 ± 5	4 ± 9
	<i>Hoplotaimus</i>	3	2 ± 3	2 ± 3	2 ± 3	3 ± 3
	Miscellaneous	3-5	9 ± 12	5 ± 11	13 ± 23	14 ± 17

^a c-p value- colonizer-persister value (Bongers, 1990).

^b Each entry represents an arithmetic mean ± standard deviation of four replicates per treatment pooled across six irrigation treatments (0.43, 0.57, 0.85, 1.00, 1.32, and 1.95 ET) (N=24). ET - evapotranspiration rate.

Table 2-2. Total abundance and trophic group composition per 100 cm³ of soil in samples taken from under the citrus trees canopy.

Trophic group	May 1995	October 1995	February 1996	May 1996
<u>Abundance</u>				
Total	461 ± 138*	253 ± 84	362 ± 143	382 ± 108
Bacterivores	296 ± 99	163 ± 73	231 ± 103	248 ± 72
Fungivores	83 ± 39	37 ± 15	79 ± 36	57 ± 24
Algivores	1 ± 3	1 ± 2	0 ± 1	0 ± 2
Predators	6 ± 7	1 ± 1	0 ± 1	1 ± 1
Plant parasites	22 ± 15	9 ± 12	18 ± 23	24 ± 21
Bacterivores and Fungivores	378 ± 117	415 ± 154	310 ± 127	306 ± 86
<u>Percentage of total numbers</u>				
Bacterivores	64.4 ± 9.0	62.5 ± 11.1	63.6 ± 8.7	65.2 ± 7.0
Fungivores	18.2 ± 6.5	15.8 ± 6.5	22.3 ± 8.0	15.0 ± 5.1
Bacterivores and Fungivores	82.6 ± 7.8	78.3 ± 10.0	85.9 ± 6.9	80.2 ± 5.6
Predators	1.3 ± 1.5	0.2 ± 0.4	0.1 ± 0.2	0.3 ± 0.4
Root associates	6.1 ± 6.5	6.0 ± 6.3	3.4 ± 2.5	3.8 ± 2.7
Plant parasites	3.8 ± 3.6	3.9 ± 5.7	5.0 ± 4.8	6.0 ± 4.5
Algivores	0.3 ± 0.6	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.4

* Data represent arithmetic means ± standard deviations of four replicates per treatments pooled across six irrigation treatments (0.43, 0.57, 0.85, 1.00, 1.32, and 1.95 ET) (N=24) on four sampling dates. ET - evapotranspiration rate.

Table 2-3. Soil moisture, levels of *Phytophthora nicotianae*, root biomass, and ecological indices obtained from soil samples taken from under the citrus tree canopy. na = no data available.

Ecological Index ^a	May 1995	October 1995	February 1996	May 1996
Soil moisture (%)	2.1 ± 0.6 ^b	4.0 ± 0.7	4.9 ± 0.5	4.4 ± 0.7
<i>P. nicotianae</i> (CFU)	0 ± 0	4.6 ± 6.7	4.7 ± 7.6	11.3 ± 15.7
Root wt (g/100g soil)	0.09 ± 0.03	0.1 ± 0.2	na	na
Richness	20.1 ± 3.3	18.9 ± 2.4	17.8 ± 2.5	20.6 ± 2.7
<i>F/B</i>	0.30 ± 0.15	0.27 ± 0.15	0.37 ± 0.17	0.24 ± 0.10
<i>F+B/PP</i>	42.7 ± 40.4	56.0 ± 67.2	30.8 ± 32.3	20.0 ± 11.8
<i>H_i</i>	1.03 ± 0.17	1.06 ± 0.17	1.00 ± 0.14	1.04 ± 0.13
λ_i	0.47 ± 0.09	0.46 ± 0.10	0.48 ± 0.09	0.47 ± 0.08
<i>H_g</i>	2.33 ± 0.19	2.30 ± 0.10	2.11 ± 0.16	2.35 ± 0.12
λ_g	0.13 ± 0.03	0.14 ± 0.02	0.17 ± 0.03	0.13 ± 0.02
$1/\lambda_g$	7.98 ± 1.92	7.38 ± 0.83	6.20 ± 1.17	8.03 ± 1.19
<i>PPI</i>	3.09 ± 0.16	3.03 ± 0.11	3.08 ± 0.15	3.04 ± 0.11

^aCFU - colony forming units (CFU/cm³ soil); Root wt - dry root weight (g/100 g dry soil); Richness - number of taxonomic units (genera); *F/B* - ratio of fungivorous to bacterivorous nematodes; *F+B/PP* - ratio of fungivorous and bacterivorous nematodes to plant parasites; *H_i* - Shannon trophic diversity; λ_i - trophic dominance; *H_g* - Shannon genus diversity; λ_g - genus dominance; $1/\lambda_g$ - Simpson's genus diversity; *PPI* - plant parasitic index.

^bEach entry represents an arithmetic average and its standard deviation of four replicates across six irrigation rates (N=24).

Table 2-4. Nematode numbers and ecological indices showing significant ($P \leq 0.05$) differences between treatments receiving smaller (0.43, 0.57, 0.85, and 1.00 ET) amounts of water and treatments receiving greater (1.35 and 1.97 ET) amounts of water. Irrigation intensity was based on historical data of evapotranspiration rate (ET).

Sampling date	Irrigation Intensity						Contrast <i>P</i> -values ^a
	0.43 ET	0.57 ET	0.85 ET	1.00 ET	1.32 ET	1.97 ET	
OMNIVOROUS GENERA (numbers/100cm ³)							
<i>Aporcelaimellus</i> (c-p 5) ^b							
May 1995	4 ± 3	4 ± 3 ^c	10 ± 4	8 ± 6	13 ± 7	14 ± 11	P < 0.05
October 1995	6 ± 2	6 ± 2	9 ± 3	6 ± 2	9 ± 7	16 ± 4	P < 0.01
February 1996	5 ± 3	6 ± 7	10 ± 13	6 ± 1	10 ± 11	12 ± 9	ns
May 1996	14 ± 19	10 ± 6	15 ± 7	12 ± 4	23 ± 14	22 ± 11	ns
<i>Eudorylaimus</i> (c-p 4)							
May 1995	5 ± 2	5 ± 2	4 ± 4	2 ± 2	11 ± 4	17 ± 8	P < 0.01
October 1995	4 ± 3	7 ± 4	6 ± 3	5 ± 5	10 ± 8	15 ± 7	P < 0.01
February 1996	6 ± 3	6 ± 7	5 ± 3	6 ± 5	10 ± 11	16 ± 10	P < 0.05
May 1996	8 ± 6	7 ± 6	9 ± 4	10 ± 3	15 ± 10	13 ± 7	ns

TOTAL OMNIVOROUS NEMATODES (numbers/100cm ³)							
May 1995	10 ± 7	18 ± 12	21 ± 4	19 ± 12	27 ± 12	40 ± 21	P < 0.01
October 1995	18 ± 3	25 ± 9	29 ± 17	15 ± 6	31 ± 16	45 ± 20	P < 0.05
February 1996	14 ± 8	13 ± 14	19 ± 16	13 ± 5	24 ± 23	33 ± 18	P < 0.05
May 1996	29 ± 25	26 ± 14	35 ± 7	26 ± 4	56 ± 25	46 ± 7	P < 0.01
OMNIVOROUS NEMATODES (as % of total nematodes)							
May 1995	2 ± 1	4 ± 3	4 ± 2	4 ± 3	6 ± 2	11 ± 4	P < 0.01
October 1995	7 ± 2	10 ± 4	11 ± 4	7 ± 1	14 ± 7	19 ± 8	P < 0.01
February 1996	4 ± 4	3 ± 3	4 ± 3	4 ± 1	6 ± 5	11 ± 5	P < 0.05
May 1996	6 ± 4	7 ± 3	9 ± 3	7 ± 2	12 ± 4	15 ± 2	P < 0.01
MATURITY INDEX (MI)							
May 1995	2.03 ± 0.06	2.10 ± 0.10	2.10 ± 0.04	2.06 ± 0.14	2.18 ± 0.16	2.29 ± 0.09	P < 0.01
October 1995	2.14 ± 0.07	2.18 ± 0.12	2.21 ± 0.09	2.11 ± 0.03	2.24 ± 0.18	2.43 ± 0.24	P < 0.01
February 1996	2.06 ± 0.10	2.05 ± 0.07	2.04 ± 0.08	2.00 ± 0.07	2.09 ± 0.16	2.23 ± 0.10	P < 0.05
May 1996	2.13 ± 0.11	2.17 ± 0.08	2.22 ± 0.08	2.17 ± 0.05	2.32 ± 0.10	2.37 ± 0.03	P < 0.01

TOTAL MATURITY INDEX (*MI*)

May 1995	2.05 ± 0.10	2.14 ± 0.13	2.15 ± 0.03	2.05 ± 0.13	2.20 ± 0.19	2.31 ± 0.13	P < 0.01
October 1995	2.16 ± 0.06	2.20 ± 0.13	2.25 ± 0.10	2.14 ± 0.04	2.33 ± 0.16	2.45 ± 0.24	P < 0.01
February 1996	2.10 ± 0.09	2.09 ± 0.05	2.11 ± 0.06	2.04 ± 0.06	2.20 ± 0.16	2.25 ± 0.09	P < 0.01
May 1996	2.19 ± 0.12	2.21 ± 0.09	2.26 ± 0.07	2.22 ± 0.05	2.38 ± 0.13	2.41 ± 0.05	P < 0.01

^a P values for the orthogonal contrast between smaller ET (0.43, 0.57, 0.85, 1.00) and greater ET (1.35, 1.97) treatments.

^b *C-p* values (colonizer-persister) taken from Bongers (1990)

^c Data represent arithmetic means ± standard deviations. Each entry was estimated using four replicates per treatment.

CHAPTER 3

RELATIONSHIP BETWEEN SOIL CHEMICAL STATUS, SOIL NEMATODE COMMUNITY, AND SUSTAINABILITY INDICES

Introduction

Agricultural management practices affect soil characteristics. Reduction in diversity of soil biota (Paoletti et al., 1992), loss of soil organic matter content, and increased soil erosion (Ehrlich, 1988) are problems in many conventional agricultural systems. Other effects of farming systems that use high amounts of matter and energy (fertilizers, pesticides, irrigation, and cultivation) are ground and surface water contamination, leading to potential hazards to human and animal health (Schaller, 1993). The above problems, along with a rapidly diminishing supply of natural resources, have forced many scientists and farmers to seek more sustainable and environmentally sound methods for food and fiber production.

Over the last decade, sustainable agriculture has become a new paradigm for the future of agriculture (Schaller, 1993; Kenneth et al., 1996). Recently, much effort has been devoted to defining chemical and biological indicators that can be useful in recognizing sustainable systems (Doran and Safley, 1997). A problem with this approach is in the definition of a sustainable agricultural system. Although many people use the term, the understanding of "sustainable agriculture" may vary from one person to another. For most people, however, sustainable agriculture symbolizes conservation of natural resources and protection of the environment as well as production of adequate amounts of food and profits for farmers on a long time scale (future generations).

Schaller (1993) argued that this definition is too broad and lacking in scientific precision. The very nature of any agroecosystem, characterized by a nearly infinite number of variables, implies that sustainability can be defined in terms of many possible variables or combinations of variables. These variables include items such as conservation of biodiversity, levels of specific pollutants and other elements, conservation of water and other natural resources, grower profits and adequate yield levels. Moreover attempts to increase sustainability of some variables may conflict with others. For example, in many regions conservation of water is desirable for future generations, but may conflict with current production practices aimed at increasing crop yields.

From the practical point of view, farmers would not likely choose to maximize either water conservation or yield, over the profitability of production practices. Moreover, the most profitable practices will be the most sustainable ones for a given set of economic conditions because of the market competition. Profitability could include broad aspects of the agroecosystem (e.g., hidden management cost such as cost of environmental remediation, projected future values of limited resources, and estimated value of biodiversity), and thereby provide a reasonable index of sustainability by revealing the optimum relationship between crop production and management inputs (Duncan and Noling, 1998). However, it is currently more feasible to restrict this definition to grower profits, and in the present study we focus on profitability and water use efficiency.

In Florida citrus orchards, irrigation is one of the most important management tactics to increase fruit yield (Jackson et al., 1995). Typical Florida soils, with very high sand content and extremely low moisture holding capacity, are exposed to constant excessive drying under conditions of relatively high temperatures. Because irrigation generally increases fruit yield, farmers may choose to apply higher than necessary amounts of water to ensure economic returns. Irrigation also affects the condition of the soil ecosystem (e.g., nutrient and pesticide loss

through leaching, accumulation of metals, and shift in microbial community structure). Detailed analysis of both chemical and biotic components of the soil ecosystem under various watering regimes can provide information about key variables (e.g., concentrations of nutrients, heavy metals, soil organic matter content, and biodiversity) that define the conditions of the soil environment. Resulting information on the soil ecosystem status may permit farmers to better manage water resources. As a biological component of the ecosystem, nematode community structure may integrate physical and chemical characteristics of the environment (Cairnes et al., 1993). Because nematodes are ubiquitous to all habitats and present a variety of feeding habits (algal-, bacterial, fungal-, omnivores, plant parasites, predators, and root associates) and life strategies (*r-K*), they are potential indicators of the status of the soil ecosystem. Nematodes have been proposed as good indicators of productivity, pollution, secondary succession, and disturbance (Bongers et al., 1991; Ferris et al., 1996; Freckman and Ettema, 1993; McSorley and Frederick, 1996; Wasilewska, 1994; Yeates, 1994; Yeates and Bird, 1994). Nematodes have also been proposed as useful in defining sustainable agroecosystems (Neher and Campbell, 1994).

The effects of soil nutrients on yields have been investigated routinely in agricultural sciences. Fewer attempts, however, have been made to look at the effects of farming practices on soil chemistry and its relationship with nematode community patterns. The objective of this study was to evaluate the long-term effects of various irrigation intensities on the nematode community and the status of several macro- and micronutrients in the soil. To validate the usefulness of nematodes as bioindicators of the soil ecosystem status, relationships between these nutrients and nematode genera and nematode community indices were investigated. In addition, we investigated nematode measures in the context of several aspects of sustainability, described here by three indices; yield, water use efficiency, and profitability.

Materials and Methods

Experimental design. The experiment was conducted at the University of Florida, Citrus Research and Education Center (CREC), Lake Alfred, Florida, and was part of the longer study. The soil was an Astatula fine sand with an average pH of 6.2 and 0.7% organic matter. For the last 80 years, the research site had been planted to citrus (*Citrus* spp.) trees. On 10 April 1991, young (approximately 1-year-old) citrus trees of 'Hamlin' orange (*Citrus sinensis* [L.] Osbeck) on 'Swingle' citrumelo (*Citrus paradisi* x *Poncirus trifoliata*) rootstock free of endoparasitic nematode pests were planted in rows 6.1 m apart, with 3.9 m between trees in rows. The irrigation treatments began on 1 June 1991, but the data presented here are from the fifth year (1996) after establishment of the experiment. During 1991 to 1996, nutrient management followed the fertilizer guidelines typical for citrus in Florida (Ferguson et al., 1995). For weed control under the tree canopies, the herbicide glyphosate was applied as necessary (3 to 4 times per year at approximately two-month intervals beginning in late February or early March). Fertilizer and herbicide rates were applied equally to all treatments.

Trees were watered at six different irrigation intensities every fourth day. Irrigation intensities were expressed as a proportion of the average evapotranspiration rate (ET): 0.43 ET, 0.57 ET, 0.85 ET, 1.00 ET, 1.32 ET, and 1.95 ET. These proportions resulted from differences in available microsprinkler size openings used in the irrigation system. The amount of water required for each month was calculated from the measurements of historical ET. For instance, if ET for January of previous 50 years was 0.18 cm of water per day, the treatment 1.00 ET would receive 0.72 (0.18 cm x 4 days) cm of water every fourth day in January. Other treatments would receive an assigned proportion of that amount (e.g. 0.43 ET would receive $0.72 \times 0.43 = 0.31$ cm of water every fourth day). The monthly ET typically varies from a low of 0.18 cm/day for January to a high of 0.46 cm/day of water in May.

The experiment was arranged in a randomized complete block design with four blocks with one replication per block. Soil samples were collected in May and October, 1995, and February and May, 1996. Each soil sample was a bulk of 16 soil cores (2 cm in diameter and 30 cm in length) taken in a diagonal transect pattern under the tree canopy. Each soil sample was passed through a sieve (2 mm x 2 mm mesh size) to separate the soil from root tissue. Samples from each sampling date were processed for nematode analysis. Samples collected on 10 February, 1996 were also processed to determine soil moisture, density of pathogenic fungi, citrus fibrous root density, and soil chemical status.

Biological measures. A subsample of approximately 100 cm³ was immediately used to estimate densities of *Phytophthora nicotianae*, a fungal pathogen of citrus roots (Timmer et al., 1988). Nematodes were extracted from another 100-cm³ subsample by wet sieving followed by centrifugation (Jenkins, 1964). Extracted nematodes were killed with heat, counted, and identified mostly to genus. Based on nematode community data, ecological indices such as fungivore to bacterivore ratio (Freckman and Ettema, 1993), fungivores plus bacterivores to plant parasites ratio (Wasilewska, 1994), richness (number of genera), trophic and generic dominance (Simpson, 1949), trophic and generic diversity (Shannon and Weaver, 1949), maturity index (Bongers, 1990), and total maturity index (Yeates, 1994) were calculated. Additional background and detailed procedures for calculation of these indices are presented elsewhere (Porazinska et al., 1998). Separated roots were washed, dried at 60° C, and weighed.

Chemical measures. A separate soil subsample of approximately 100 cm³ was air-dried and used to determine various soil chemical properties. Mehlich-I extractable Ca, Mg, K, Na, P, Cu, Fe, Mn, and Zn (Mehlich, 1953) as well as N, pH, buffer pH, soil organic matter (SOM), and cation exchange capacity (CEC) were determined. Calcium, Mg, Cu, Fe, Mn, and Zn concentrations in soil extracts were determined by atomic absorption spectrophotometry. Potassium and Na were resolved by atomic emission spectrophotometry using a Perkin-Elmer

Atomic Absorption Spectrophotometer. Concentrations of P were estimated by colorimetry. Soil water pH was evaluated from 20 ml of soil suspended in 40 ml of deionized water. The pH measurements were taken 30 minutes after stirring, with a calibrated pH-meter. Soil organic matter was estimated (modified procedure of Jackson, 1958 and Horwitz, 1975) by placing 1 g of soil in a 500-ml Erlenmeyer flask, adding 10 ml of 1 N K_2SO_4 and mixing by gentle rotation for about one minute. The mixture was diluted with deionized water to 200 ml after 30 minutes, and 5 drops of an indicator were added. The suspension was titrated with 0.5 N $FeSO_4$ until the dull green color of the solution changed to a reddish brown. A soil sample with a known organic matter content was utilized as a check sample. CEC was determined by cation summation (Jackson, 1958). An aluminum digest block was used to capture N in soil extract (Gallaher et al., 1975). A Technicon Autoanalyzer II was used to determine concentrations of N (N was trapped and recorded as $(NH_4)_2SO_4$).

Sustainability measures. Yield, water use efficiency, and profitability were measured. Because the trees have not reached the productive maturity, the yield data were presented in three forms: separate fruit production for 1995 and 1996, and 1995 and 1996 pooled into one fruit yield value to better illustrate tree fruit productivity. Water use efficiency (kg/cm), which expresses yield per centimeter of irrigation water applied, was derived from the yield (kg/ha) data divided by the amount of water used in a particular treatment (cm/ha). Profitability was based on a typical cost-benefit analysis used by farmers. Costs were estimated using budget tables created by Muraro (1997). All cost factors were constant across irrigation treatments, except fuel cost for irrigation pumps, which varied in proportion to the six water treatment levels. We assumed a price for a box of fruit (40.9 kg) to be \$5.60 (Timmer, 1997).

Statistical analysis. Relationship between irrigation intensity and soil chemical properties, nematode community measures, *Phytophthora nicotianae* populations, root biomass, and soil moisture on 10 Feb, 1996, were derived using correlation analysis. Selected

relationships were further examined using analysis of variance and orthogonal contrasts procedures. All statistical computations were performed utilizing SAS software (SAS Institute, Inc., Cary, NC). Selected relationships involving nematode measurements or indices were also examined using data from the remaining sampling dates.

Results and Discussion

In the context of sustainable agriculture, the information on various components of the soil ecosystem becomes crucial. It has been suggested that only detailed information about the entire ecosystem may allow us to understand managed systems more thoroughly and thus improve management decisions. The effects of soil nutrients on yields have been investigated in agricultural sciences routinely. Fewer attempts, however, have been made to look at the effects of farming practices on soil chemistry and its relationships with nematode community patterns. Given the assumption such relationships are revealed, nematode indices could be useful in designing more sustainable agricultural management programs. It is important to emphasize here that chemical soil description fails as an indicator of soil conditions for many reasons (many ions are transient and unstable making them difficult for measurements, concentrations do not address the issue of bioavailability or biotoxicity, the effects of natural elements on soil biota are mostly unknown, etc.). Clearly, a biotic component of the soil environment (nematodes) might better serve the purpose of a good indicator of the soil status.

Chemical measures. The data presented here are from the fifth year of the experiment, and thus should reflect the longer-term effects of different irrigation rates on the soil chemistry. A significant ($P \leq 0.05$) positive correlation with the irrigation rate was found for Ca, Mg, Zn, pH, and buffer pH (BpH) ($r = 0.48$ for Ca, $r = 0.56$ for Mg, and $r = 0.48$ for Zn) (Table 3-1). While Fe was not correlated with the irrigation rate, the orthogonal contrasts procedure revealed that the highest concentrations of Fe were associated with treatments receiving the lowest

amounts of water (0.57, 0.43 ET) and were significantly higher ($P \leq 0.05$) than Fe concentrations in treatments with the four higher water rates.

Soil pH was positively correlated with the increase of irrigation intensity ($r = 0.79$, $P \leq 0.05$) (Table 3-2). In the lowest water rate treatment, the soil was slightly acidic and pH steadily increased until reaching almost neutrality in the treatment receiving the highest amount of water. Irrigation intensity was also positively correlated with buffer pH ($r = 0.64$, $P \leq 0.05$).

Various water level treatments affected only some soil chemical measurements. The shift of Ca and Mg concentrations in the soil with the water rate increase can be explained by Ca and Mg present in the irrigation water derived from subsurface wells in soil horizons rich in limestone and dolomites (Brown et. al., 1991). High levels of Ca and Mg in soil help to keep soil pH close to neutral. Increasing soil pH to slightly below 7 has been associated with consistent and significant citrus yield improvements (Jackson et al., 1995).

Accumulation of Zn in the soil may pose a toxicity threat to citrus trees. Interestingly, concentrations of Zn increased as more water was applied (higher pH). Generally, in situations of high Cu, Mn, or Zn levels, additions of higher Ca amounts should suffice to control the levels of these heavy metals in the soil. Declining Fe levels with irrigation elevation suggest that either Fe became unavailable due to higher pH levels or Fe was leached to the deeper layers of the soil profile.

Lack of consistent patterns for P and N in relation to water regimes may be explained by high immobility of P in the soil (Myrold, 1998; Mullen, 1998), and by the N management practice which involves 6 to 7 applications of this fertilizer per year (Ferguson et al., 1995). The very low soil organic matter reflected the extremely high sand content (up to 98%) and continuous control of weeds by herbicides under the tree canopy. Only a small fraction of plant debris reaches the soil surface and thus limits the possibilities for soil organic matter formation.

Biological measures. Populations of *P. nicotianae* and root biomass did not have any linear relationship with soil nutrients. Only 30 to 40 % of all nematode genera and half of the nematode community indices were correlated with the chemical elemental levels (Table 3-2). *Acrobeloides*, *Cephalobus*, and *Eucephalobus* (bacterial feeders) belong to the same nematode family (Cephalobidae); however, they responded differently to soil chemical characteristics, indicating their preferences to different soil microhabitats. Moreover, the implications of correlations are unclear. The extent of ecological knowledge on bacterivorous nematodes is still very limited and requires further investigation. At this stage of knowledge it would be unwise to suggest bacterivores as definite and dependable indicators of sustainability attributes.

Among the plant parasites, *Belonolaimus longicaudatus* can cause severe problems on citrus trees (Duncan et al., 1996). From the economic perspective, information about this nematode is of particular interest. Densities of *Belonolaimus* were negatively correlated with pH and Mg concentrations and are thus related to the irrigation rate. Whether population density is regulated by water, chemical properties or another variable is unknown.

The majority of omnivorous nematodes are *K*-strategists (long life cycle, low reproductive rate, few offsprings, stable habitats); therefore, they are believed to be sensitive to environmental stressors (Bongers, 1990). Presence of high numbers of omnivores in the soil should indicate stable soil systems. Population densities of the omnivore genera *Eudorylaimus*, *Aporcelaimellus*, total numbers of omnivores, and the ratio of omnivores to total abundance (O/T), all showed similar patterns, usually favoring high pH, BpH, and high Ca and Mg concentrations. Since these measures were highly correlated with the irrigation intensity (Porazinska et al., 1998), it would be important to investigate the nature of this relationship in more detail. Relatively high correlations of *Eudorylaimus* and *Aporcelaimellus* with Zn and Mn concentrations suggest that these metals may be far from approaching any toxic concentration levels and thus control of micronutrient availability by liming is unnecessary.

Maturity index (*MI*) and total maturity index ($\sum MI$) (Bongers, 1990; Yeates, 1994) were related to many of the same variables as omnivorous nematodes. This response was expected since an increase of *MI* and $\sum MI$ values usually results from higher proportions of omnivores in the nematode community.

A useful bioindicator of environmental status should probably be responsive to a wide range of factors. In the current study, the nematode genera showing significant correlations with the most chemical elements were *Criconemoides* (6 elements), *Eucephalobus* (3 elements), *Plectus* (3 elements), *Prismatolaimus* (3 elements), and *Wilsonema* (3 elements). Of all the factors measured (Table 3-2), *Criconemoides* correlated with 7, *Prismatolaimus* with 5, and *Acrobeloides*, *Eucephalobus*, *Aporcelaimellus*, and total omnivores with 4 each. Of course, the utility of this approach, also requires many additional data and improved knowledge of nematode biology.

Sustainability measures. In general, higher irrigation levels positively influenced citrus tree fruit production (Figure 3-1). Profitability estimates indicated that 1996 was the first year of monetary gains in this citrus orchard. This is a rather typical scenario because it takes about 7-10 years for a citrus tree to reach its productive maturity. Cumulative profitability suggested that the biggest financial losses were associated with the lowest water treatments (Figure 3-2). However, greater than 0.85 ET irrigation levels did not seem to contribute to minimization of those losses. In 1996, we observed a similar trend, with the 0.85 ET as the most profitable water treatment. Therefore, slightly greater yield recovery from treatments higher than 0.85 ET water levels, did not justify the management (irrigation) expenses. This would be even more obvious if a minimal price of water was added to the management costs (e.g. 0.0002 cent/l), under which scenario the 0.85 ET treatment is the only profitable one. As suspected, water use efficiency was lowest in treatments receiving the greatest amounts of water, indicating poor water conservation efforts at the highest rates (Figure 3-3).

Few nematodes had relationships with all the measures of sustainability presented here (Table 3-3). *Prismatolaimus*, an unusual bacterivore (*K*-strategist), seems to be particularly interesting, indicating several aspects of sustainability at the same time. From the economic standpoint (here represented by yield and profitability), treatments with lower densities of *Prismatolaimus* (higher water rates) appear favorable, whereas, treatments producing highest densities of *Prismatolaimus* contribute to water conservation. Very similar relationships were observed for *Eucephalobus* and *Belonolaimus*.

In contrast, omnivores, % omnivores, *MI*, and *TMI* exhibited inverse relationships with water use efficiency. By virtue of representing broad groups of species, these latter categories are likely to be more robust indicators of environmental status because, unlike individual species, they can be estimated from all soil samples. Indeed, % omnivores, *MI*, and *TMI* were also inversely related ($P \leq 0.05$) to water use efficiency on each of the other sampling dates. The mean (and standard error) correlation coefficients for % omnivores, *MI*, and *TMI* with water use efficiency (1995 and 1995 + 1996) for all sampling dates were -0.61 (0.05), -0.53 (0.03), and -0.53 (0.04), respectively. No other community index, nor any single species was so closely related to water use efficiency, considering the magnitude of correlation coefficients or the number of dates on which the relationship was significant.

In this study, different irrigation treatments were required to maximize either water use efficiency or profitability. Consequently, biological indices revealed the status of one or the other sustainability index, or alternated between positive and negative correlations for one or the other. Because irrigation directly affects the soil ecology, it is not surprising that the biological indices were more reflective of water use efficiency (irrigation rate) than profitability. Nevertheless, agricultural systems are more or less sustainable based on profitability as well as on resource availability and management. Developing rational management practices will require prioritizing these requirements. Water use efficiency should have a higher social priority

than profitability in regions where natural recharge of water does not exceed its use. Various means (water surcharges, water use restrictions, etc.) are available to society to increase water use efficiency in agricultural and other endeavors. However, it is desirable to minimize the use of these means because they come at a cost to society in the form of higher food prices. For this reason, profitability that includes not only production but also “environmental” costs, remains an important consideration when prioritizing factors related to agricultural sustainability.

Information on the relationship between the irrigation regimes and the chemical soil status and its relationship with the nematode community, can illustrate many aspects of the soil condition. In terms of water conservation, several chemical and nematode indices reflected some of the soil conditions (treatments receiving greater amounts of water) of this ecosystem. No relationship between the majority of nematode indices and profitability were found, indicating that this condition of sustainability often has little biological basis. Since yield, water use efficiency, and profitability reflect different aspects of sustainable agriculture, they may conflict with respect to optimum management practices. In our experiment, however, based on the revealed relationship between *K*-strategists and water use efficiency, and between irrigation level and profitability, we suggest 0.85 ET as the best soil water regime for most components of sustainable agriculture, and omnivorous nematodes as the most useful indicators of water management history.

Table 3-1. Soil chemical measures obtained from soil beneath citrus trees exposed to six different irrigation intensities expressed as a proportions of the evapotranspiration rate (ET) (0.43, 0.57, 0.85, 1.00, 1.32, and 1.97 ET) on 10 February 1996. The concentrations of soil minerals are in mg/kg. Bph = buffer pH, CEC = cation exchange capacity in meq/100g, OM = organic matter in %.

Measurement	Irrigation Intensity					
	0.43 ET	0.57 ET	0.85 ET	1.00 ET	1.32 ET	1.97 ET
Ca	262 ± 85	271 ± 76	224 ± 59	278 ± 110	289 ± 49	397 ± 121
Mg	19.4 ± 3.7	21.4 ± 2.1	22.1 ± 5.8	20.3 ± 2.7	25.5 ± 4.1	27.2 ± 5.1
K	13.0 ± 3.8	12.3 ± 2.5	12.2 ± 2.3	9.4 ± 1.3	12.2 ± 3.5	10.7 ± 0.7
Na	22.1 ± 4.0	18.6 ± 3.2	17.0 ± 1.5	19.6 ± 4.5	18.0 ± 0.8	19.7 ± 4.2
N	274 ± 15	296 ± 58	290 ± 67	243 ± 11	287 ± 50.0	296 ± 23.4
P	91.3 ± 28.6	81.6 ± 30.1	53.2 ± 8.1	87.2 ± 42.5	57.6 ± 7.2	91.7 ± 34.2
Cu	16.5 ± 6.8	14.6 ± 4.4	15.7 ± 8.0	16.4 ± 6.7	15.2 ± 2.9	15.8 ± 3.7
Fe	19.2 ± 1.5	17.1 ± 1.7	15.4 ± 2.9	15.7 ± 0.8	15.0 ± 1.7	16.6 ± 0.5
Mn	10.16 ± 1.79	9.10 ± 1.28	9.24 ± 2.21	8.70 ± 3.31	8.85 ± 1.25	9.44 ± 0.88
Zn	3.88 ± 2.10	5.08 ± 2.40	4.44 ± 1.67	4.69 ± 2.35	5.62 ± 0.98	6.96 ± 1.23
pH	5.45 ± 0.13	5.95 ± 0.17	6.10 ± 0.29	6.30 ± 0.39	6.38 ± 0.13	6.68 ± 0.30
BpH	7.88 ± 0.01	7.93 ± 0.02	7.93 ± 0.05	7.95 ± 0.03	7.95 ± 0.03	7.97 ± 0.01
CEC	2.56 ± 0.50	2.25 ± 0.31	1.95 ± 0.73	2.05 ± 0.72	2.17 ± 0.47	2.59 ± 0.65
OM	0.62 ± 0.07	0.70 ± 0.03	0.60 ± 0.27	0.52 ± 0.10	0.38 ± 0.34	0.84 ± 0.27

Data represent means ± standard deviations of four replicates.

Table 3-2. Significant (at $P \leq 0.10$) correlation coefficients between soil chemical measures, nematode genera, and nematode community indices. BpH = buffer pH, CEC = cation exchange capacity, SOM = soil organic matter, B/F = bacterivorous to fungivorous nematode ratio, B/T = proportion of bacterivorous nematodes in the nematode community, (B+F)/T = proportion of bacterivorous and fungivorous nematodes together in the nematode community, O/T = proportion of omnivorous nematodes in the nematode community, trophic div. = trophic diversity, trophic dom. = trophic dominance (Freckman and Etema, 1994), ΣMI = maturity index (Yeates, 1993), MI = maturity index (Bongers, 1990).

Measurement	Ca	Mg	K	Na	N	P	Cu	Fe	Mn	Zn	pH	BpH	CEC	% SOM
<u>Bacterial feeders</u>														
<i>Acrobeloides</i>	-	-	0.459**	-	-	-	-	0.409**	-	-	-0.390*	-0.368*	-	-
<i>Cephalobus</i>	-	-	0.402**	-	-	-	-	-	-	-	-	-	-	-
<i>Eucephalobus</i>	-	-0.453**	-	-	-	-	-	-	-	-	-0.491**	-0.501**	-	-
<i>Plectus</i>	0.414**	0.472**	-	-	-	-	-	-	-	-	0.437**	-	-	-
<i>Prismatolaimus</i>	-	-	-	0.364*	-	-	-	0.537**	0.355*	-	-0.553**	-0.537**	-	-
<i>Wilsonema</i>	-	0.436**	-	-	-	-	-	-	-	-	0.456**	0.502**	-	-
<u>Fungal feeders</u>														
<i>Aphelenchoides</i>	-	-	-	-	-	-	-	0.492**	-	-	-	-	-	-
<u>Omnivores</u>														
<i>Eudorylaimus</i>	0.413**	0.485**	-	-	-	-	-	-	-	0.387*	-	-	-	-
<i>Aporcelaimellus</i>	-	-	-	-	-	-	-	-	0.368*	0.381*	0.368*	0.381*	-	-
<u>Root associates</u>														
<i>Tylenchus</i>	-	-	0.543**	-	-	-	-	-	-	-	-	-	-	-0.425**
<u>Plant parasites</u>														
<i>Belonolaimus</i>	-	-0.433**	-	-	-	-	-	-	-	-	-0.377*	-	-	-

<i>Criconemoides</i>	-	0.379*	-	0.503**	-	0.522**	-	0.424**	0.423**	-	-0.453**	0.490**	-
Total	-	-	0.367*	-	-	-	-	-	-	-	-	-	-
Bacterivores	-	-	0.375*	-	-	-	-	-	-	-	-	-	-
Fungivores	-	-	-	-	0.371*	-	0.345*	-	-	-	-	-	-
Omnivores	0.353*	0.391*	-	-	-	-	-	-	0.424**	0.421**	-	-	-
B/F	-	-	0.363*	-	-	-	-	-	-	-	-	-	-
B/T	-	-0.456**	-	-	-	-	-	-	-	-	-	-	-
(B+F)/T	-	-	-	-	-	-	-	-	-	-	-	0.418**	-
O/T	0.366*	-	-	-	-	-	-	-	0.435**	0.423**	-	-	-
H ₂ O	-	0.489**	-	-	0.391*	-	-	-	0.403*	-	-	-	-
Richness	-	0.353*	-	-	-	-	-	-	-	-	-	-	-
Trophic div.	-	0.442**	-	-	-	-	-	-	-	-	-	-	-
Trophic dom..	-	-0.448**	-	-	-	-	-	-	-	-	-	-	-
ΣMI	-	0.478**	-	-	-	-	-	-	0.362*	-	-	-	-
<i>MI</i>	0.369*	0.359*	-	-	-	-	-	-	-	-	-	-	-
Irrigation ^z	0.484**	0.560**	-	-	-	-	-	-	0.476**	0.793**	0.640**	-	-

** statistically significant at $P \leq 0.05$

* statistically significant at $P \leq 0.10$

^z Irrigation intensity as proportion of evapotranspiration rate.

Table 3-3. Significant (at $P \leq 0.10$) correlation coefficients between chemical or biological measures and indices of sustainability. BpH = buffer pH, B/T = proportion of bacterivorous nematodes in the nematode community, B+F/T = proportion of bacterivorous and fungivorous nematodes in the nematode community, O/T = proportion of omnivorous nematodes in the nematode community, H_i = trophic diversity, λ_i = trophic dominance, ΣMI = maturity index, MI = maturity index.

Measurement	Yield 96	Yield 95+96	Water use efficiency 96	Water use efficiency 95+96	Profitability 96	Profitability 95+96
Ca	-		-0.420**		-	
Mg	-		-0.525**		-	
Na	-0.427**		-		-0.442	
P	-		-		-0.355*	
Fe	-0.650**		0.432**		-0.638**	
Zn	-		-0.435**		-	
pH	0.642**		-0.822**		0.545**	
BpH	0.602**		-0.682**		0.530**	
Monhysterida	-0.570**	-0.552**	0.374*	0.368*	-0.553**	-0.555**
<i>Eucephalobus</i>	-0.480**	-0.521**	0.498**	0.497**	-0.427**	-0.487**
<i>Prismatolaimus</i>	-0.471**	-0.529**	0.412**	0.389*	-0.436**	-0.518**
<i>Wislonema</i>	-	-	-0.440**	-0.437**	-	-
<i>Eudorylaimus</i>	-	-	-0.424**	-0.413**	-	-
<i>Belonolaimus</i>	-	-0.480**	0.470**	0.435**	-	-0.444
Omnivores	-	-	-0.407**	-0.405**	-	-
B/T	-	-0.371*	-	-	-	-0.371*
B+F/T	-	-	-	0.382*	-	-
O/T	-	-	-0.487**	-0.489**	-	-
H_i	-	0.363*	-	-	-	0.347*
λ_i	-	-0.368*	-	-	-	-0.367*
MI	-	-	-0.479**	-0.466**	-	-
MI	-	-	-0.406**	-0.400*	-	-
Soil moisture	-	0.379*	-0.440**	-0.426**	-	0.350*

** statistically significant at $P \leq 0.05$

* statistically significant at $P \leq 0.10$

CHAPTER 4

NEMATODE COMMUNITIES AS INDICATORS OF STATUS AND PROCESSES OF A SOIL ECOSYSTEM INFLUENCED BY AGRICULTURAL MANAGEMENT PRACTICES

Introduction

Several characteristics of soil nematodes make them good candidates for bioindicators of the status and processes of an ecosystem. Nematodes possess the most important attributes of any prospective bioindicator (Cairnes et al., 1993): abundance in virtually all environments, diversity of life strategies and feeding habits (Freckman, 1988; Yeates et al., 1993a), short life cycles, and relatively well defined sampling procedures. For these reasons several researchers have attempted to develop relationships between nematode community structure and succession of natural ecosystems or environmental disturbance (Ettema and Bongers, 1993; Freckman and Ettema, 1993; Freckman and Virginia, 1997; de Goede et al., 1993; Wasilewska, 1994; Yeates and Bird, 1994). In general, nematode communities or ecological indices derived from them reflected differences between undisturbed and human-impacted environments.

Conventional farming systems have been associated with many environmental ills. Common problems such as loss of soil fertility, soil erosion, reduction of soil biodiversity, and ground water pollution (Ehrlich, 1988) may become crucial issues affecting production of sufficient amounts of food and fiber. A key to success of sustainable agriculture (Schaller, 1993) will be conservation of natural resources and higher dependence on natural ecosystem processes (Elliot and Colle, 1989).

The ability to monitor and assess the quality of agroecosystem soils would be of significant importance, particularly to farm managers, who could modify their farming strategies

accordingly. The idea that changes in the soil environment imposed by agricultural management practices could be revealed by measures of nematode community patterns has been investigated in recent years (Ferris et al., 1996; Freckman and Ettema, 1993; McSorley and Frederick, 1996; Porazinska et al., 1998; Yeates et al., 1997). The more challenging task still to be undertaken is interpretation of those patterns. For instance, quantification of diversity or maturity indices for simple ecosystems comparisons is of limited value. An assumption that reduced diversity negatively affects stability and thus sustainability of agroecosystems needs to be validated. Components of sustainability of agroecosystems and natural ecosystems may differ. For example, Porazinska et al. (1998a, in press) found maturity indices of nematode communities positively correlated with irrigation levels, but negatively correlated with fruit production and profitability of a citrus orchard in Florida, U.S.A. Higher maturity indices indicated environments suitable for nematode *K*-strategists, but were achieved through overuse of water resources. Thus, it is important to find trends illustrating the soil condition, but more important to find their cause. Only this type of information would allow us to use our knowledge of a relationship between soil biodiversity and some measures of an ecosystem (energy use, disturbance, profitability, or resource conservation) to increase its sustainability.

The objectives of this study were to: 1) characterize nematode communities (taxonomic and ecological index description) in soils exposed to different agricultural management practices (under same physico-chemical soil properties, vegetation cover, and climatic conditions); 2) evaluate whether soil ecosystem differences imposed by farming tactics can be reflected by nematode characterization (key taxa or indices); 3) determine which measures of ecological characterization are the most useful in differentiating various agricultural regimes; and 4) determine which of the ecological measures most adequately illustrate the status and processes of the soil environment.

Material and Methods

Site description. The experimental site was located on the property of the University of Florida Citrus Research and Education Center (CREC) in Lake Alfred, Florida (28°6'N, 81°45'W). The soil is characterized as Astatula fine sand with pH 6.2 and 1.2 % organic matter. The experimental site had been previously (70 years) planted with citrus trees (*Citrus* spp.). In 1986 young citrus trees of 'Hamlin' orange (*Citrus sinensis* (L.) Osbeck) on 'Cleopatra' mandarin (*C. reticulata*) were planted in rows 6 m apart with 4.6 m between trees within rows. On 2 February 1995, 32 trees of similar size (5 m height) and vigor were chosen and preliminary soil samples collected to determine a baseline data. A 2 x 2 x 2 factorial experiment was established, involving two fertilization levels, two irrigation levels, and two types of ground cover under the tree. The eight treatments combinations were replicated four times, in four blocks based on densities of the plant parasites *Belonolaimus* spp. and *Phytophthora nicotianae* Breda de Haan (Timmer et al., 1988) in the preliminary soil samples. Individual plots (one tree per plot) were fertilized either with typically used (336 kg N/ha/year) or reduced amount of nitrogen (168 kg N/ha/year) divided over six applications: February, March, April, May, August, and October. The N was applied as a granular 8:4:8 (N:P₂O₅:K₂O) fertilizer. For the reduced nitrogen treatment, phosphorus and potassium applications were also reduced to maintain the 8:4:8 ratio (changes of the N:P:K ratio interfere with N uptake by citrus roots). Water was delivered to trees through a microsprinkler irrigation system. Plots receiving the standard amount of water were irrigated twice a week, typically on Mondays and Fridays during spring, summer, and fall (except raining days), and once a week in winter. The duration of irrigation was always the same. When the water potential (at depth of 15 cm in the soil profile) in plots receiving the reduced amount of water would drop to approximately -15kPa, trees would be irrigated in the same manner as the trees in the standard water treatments. To control the weed growth under the tree canopies, plots received either the standard herbicide glyphosate (N-

(phosphonomethyl) glycine, isopropylamine salt) or mulch (alternative to herbicide practice). Generally, glyphosate was applied as 2.2 kg ai in 467.7 L water/ha three times a year: March, June, and October. Mulch was obtained from Bedmister Bioconversion Inc., Sevierville, TN. The mulch contained 90% municipal waste compost with almost no visible inert material (glass, plastic, etc.) and 10% wastewater residual (Widmer et al., 1997). The compost was high in calcium and thus slightly alkaline (Table 4-1). The C:N ratio was 19.3. Although heavy metal concentrations seemed to be relatively high, the municipal compost waste met the U.S. Environmental Protection Agency's criteria for exceptional quality (U.S. EPA, 1990). A layer of about 10 cm (approximately 75 t/ha) of mulch was spread evenly under the tree canopy (from tree stem to tree drip line) on 4 April 1995. The same procedure was repeated approximately two years later on 27 May 1997.

Sampling and Extraction. Soil samples were collected at approximately three-month intervals for three years (11 times): 2 February 1995 (baseline), 8 May 1995, 19 September 1995, 10 February 1996, 9 May 1996, 3 September 1996, 19 December 1996, 1 April 1997, 3 June 1997, 26 September 1997, and 21 December 1997. Each soil sample consisted of 16 cores (2-cm in diameter and 30-cm length) taken in a diagonal transect pattern from under the tree canopy. Immediately before insertion of the core into the soil profile, mulch was moved to expose the soil surface. Mulch was replaced once soil sampling was accomplished. The soil cores were mixed and passed through a sieve (2 mm by 2 mm mesh size) to separate the roots from the soil. A soil subsample of approximately 100-cm³ was used immediately to estimate populations of *Phytophthora nicotianae*, a pathogen causing citrus root decay (Timmer et al., 1988). The remaining soil was transported to Gainesville, FL in sealed plastic bags and then stored at 10°C for no longer than 15 hours. Nematodes were extracted from 100-cm³ soil subsamples by wet sieving followed by centrifugation (Jenkins, 1964). The extracted nematodes

were killed with heat (4-5 min at 60°C), identified to genus, and counted under an inverted microscope.

To estimate root biomass, citrus roots were separated from soil, washed and dried in an oven for about 96 hours at 60°C. From each soil sample, 4 to 8 g of soil were dried to constant weight in an oven at 60°C to determine soil moisture. Fungal and bacterial population densities in soils collected on 1 April 1997, 3 June 1997, and 26 September 1997 were estimated using a dilution plate method (Casida, 1968; Johnson and Curl, 1972). Yearly fruit yield was determined from citrus harvests taken on 6 December 1995, 13 February 1997, and 15 January 1998.

Nematode measures. The total number of nematodes in each genus, the total number of nematodes of different genera, and the total number of genera (richness) were recorded for every soil sample. All nematode genera were assigned to seven trophic groups: algivores, bacterivores, fungivores, omnivores, plant associates, plant parasites, and predators (Yeates et al., 1993a). The total number of nematodes in every trophic group and the percentage of every trophic group within the nematode community were also recorded.

Based on the densities of genera and trophic groups, ecological indices of the nematode community were derived. The Shannon-Weaver diversity index (1949) was used to compare diversity of either genera or trophic groups, and Simpson's (1949) index was used to compare either generic or trophic dominance. An additional measure of diversity was derived from a reciprocal transformation of the Simpson's index (Freckman and Ettema, 1993). Maturity indices *sensu* Bongers (1990) and *sensu* Yeates (1994) were also calculated. Maturity index is a semi-quantitative measure since it takes into consideration biological and ecological characteristics of individual nematode species comprising a particular community. Nematodes at the family level are ranked on a *c-p* scale from 1 (colonizer) to 5 (persister) illustrating their life and feeding strategies and, presumably, the conditions of the surrounding environment (Bongers, 1990). Maturity index is calculated as a weighted mean of the *c-p* values. Bongers defines two

types of maturity indices: *MI* - includes nematodes belonging to all feeding types except herbivores, and *PPI* - includes herbivores only. Yeates combines those two into one total maturity index – *TMI*. In general, the higher a maturity index value, the more stabilized the ecosystem. Fungivore-to bacterivore-ratio (Freckman and Ettema, 1993) and fungivores + bacterivores to plant parasites ratio (Wasilewska, 1994) were calculated to compare decomposition and nutrient mineralization pathways and primary production. More details on derivation of all the above ecological indices are given elsewhere (Porazinska et al., 1998).

Statistical analysis. The effects of different agricultural practices (two levels of fertilizer, two levels of irrigation, and two levels of ground cover) on the measures of nematode genera and community structure were determined by repeated measures - split-plot in time analysis using SAS software (SAS Institute, Cary, NC). In addition, canonical discriminant analysis on taxonomic description of nematode communities was performed for treatment separation.

Results

While water and fertilization levels only occasionally affected a selected number of nematode genera and nematode community indices, mulch had more consistent and frequent effects on many nematode measures throughout the entire time of the experiment (Table 4-2). Discriminant analysis confirmed the univariate analysis results. The first canonical explained 35 % of the total variation and separated mulch from herbicide treatments (Figure 4-1). For the above reason, this paper will emphasize the influence of compost additions on the nematode community structure.

Nematode populations. Total numbers of nematodes ranged from 359 to 821 per 100cm³ in treatments without mulch, and from 412 to 1396 per 100cm³ in treatments with mulch additions (Figure 4-2a). Within mulch treated plots, total numbers of nematodes always increased 3-4 weeks after mulch application. After the first mulch addition, the effect of

compost on total nematode numbers was very brief and lasted less than 3 months. The second mulch treatment in 1997 resulted in significantly ($P \leq 0.05$) higher nematode numbers for at least 7 months (three consecutive sampling occasions). This pattern was driven mostly by bacterivorous and fungivorous nematodes (Figure 4-2b-c).

Bacterivores dominated nematode communities in both mulch and no mulch treatments and their contribution to the total nematode community ranged between 48 and 76% (data not shown). Bacterial feeders showed an immediate but short-lived numerical response to mulch additions (Figure 4-2b). Bacterial-feeding taxa significantly contributing to this trend were Rhabditidae and *Cephalobus* (Figure 4-3a,b). *Acrobeles*, *Acrobeloides*, *Eucephalobus*, and *Teratocephalus* were either always or almost always more abundant in mulch-free plots (Figure 4-3c-f). *Plectus*, however, was always more numerous in mulch-treated plots (Figure 4-3g). *Wilsonema* tended to prefer mulch-influenced environments from the midpoint of the experiment (Figure 4-3h). Other bacterivores (*Alaimus*, *Chronogaster*, *Heterocephalobus*, *Monhystera*, *Prismatolaimus*, and *Zeldia*) were not affected by mulch treatments.

Fungivorous nematodes were more abundant in mulch-treated plots but only after the second compost addition (May 1997) (Figure 4-2c). This effect was detectable for at least 7 months until December 1997. A similar relationship was observed when fungivores were expressed as a proportion of the entire nematode community (Figure 4-2e). *Aphelenchus* was the dominant fungivorous genus and thus responsible for the shape of the trend at the trophic group level (Figure 4-4a). *Aphelenchoides* was much less abundant, however, it responded to both mulch additions, increasing in numbers immediately after placement of mulch under the trees in May 1995 and June 1997 (Figure 4-4b). These population peaks were short-lived because within next three months the population numbers were comparable to those found in mulch-free soils. Other fungal feeders such as *Diphterophora* and *Tylencholaimellus* were rather sporadic and thus no relationship could be detected.

Bacterivores and fungivores taken together as the nematodes associated with decomposition, showed a quick increase in abundance upon mulch addition, however, with time their numbers decreased, even below the levels found in treatments without mulch in May 1996. The second mulch application resulted in a significant ($P \leq 0.05$) and more prolonged response of decomposers (Figure 4-2g).

Omnivorous nematodes, whether expressed as numbers or percentage of the total nematodes, were nearly always more common in treatments not exposed to mulch (Figure 4-2d,f). Omnivores were represented by *Aporcelaimus*, *Aporcelaimellus*, *Ecumenicus*, *Eudorylaimus*, *Mesodorylaimus*, and *Pungentus*. Although not always statistically significant ($P \leq 0.05$), *Eudorylaimus* was always more numerous (Figure 4-5a) and *Aporcelaimellus* (Figure 4-5b) almost always more numerous in plots without mulch.

Plant-parasitic nematodes made up 6-20 % of the nematode community, with numbers ranging from 35 to 256 per 100 cm³ (data not shown). As a trophic group, plant parasites did not respond to compost treatment in any predictable manner. However, several genera showed consistent patterns. While *Belonolaimus* was usually more numerous in plots with mulch (Figure 4-6a), *Criconemoides* was usually less numerous (Figure 4-6b). Other plant parasites (*Hoplolaimus*, *Meloidogyne*, *Pratylenchus*, *Tylenchulus*, *Trichodorus*, and *Xiphinema*) did not reveal significant patterns.

Tylenchidae (predominantly *Tylenchus* spp.) were considered as root associates since their feeding habits are still very controversial. Within first half of the experiment these nematodes were more numerous in soils without mulch, but after the second mulch application the pattern reversed (Figure 4-6c).

Predators and algivores were uncommon in our soils and on many occasions were totally absent. On average, their numbers ranged between 0 and 5 per 100 cm³ soil (data not shown).

Ecological indices. Diversity indices such as richness (total number of genera), Shannon-Weaver trophic diversity, Shannon-Weaver generic diversity, and Simpson's diversity were usually higher in treatments free of mulch (Figure 4-7a-d). The index of dominance (at the generic level) showed the nematode communities more dominated by few abundant genera in mulch treated soils (Figure 4-7e). The ratio of bacterivores and fungivores to plant parasites ($B+F/PP$) was typically higher in plots treated by compost especially during the second half of the experiment (Figure 4-8a). Fungivore to bacterivore ratio (F/B) (Figure 4-8b) could differentiate mulch from non-mulch treatments only after the second mulch addition. From June 1997 to December 1997, F/B was significantly ($P \leq 0.05$) higher in soils exposed to municipal waste compost. The maturity indices were nearly always greater in plots without mulch (Figure 4-9a-c).

Other measured variables. Soil moisture was always higher in soils covered with mulch (Figure 4-10a). *Phytophthora nicotianae* was temporally variable and was not suppressed by compost treatments. On two occasions, the numbers of *P. nicotianae* were significantly ($P \leq 0.05$) higher in mulched plots (Figure 4-10b). Although not significantly different, total fungal populations in soils receiving mulch were slightly higher than in soils treated with herbicide (40 and 36×10^2 CFU / g dry weight soil respectively) in June 1997 (a week after mulch application). Three months later, however, the scenario was reversed (25 and 34×10^2 CFU / g dry weight soil).

Mulch had a positive effect on fruit yields. Every year, significantly ($P \leq 0.05$) more fruit yield (28 to 33%) was harvested from trees treated with mulch (Figure 4-10c).

The mulch layer under the tree canopy was not effective in controlling weeds. Usually they were so abundant that the ground surface under the tree was not visible. The plots treated with herbicide occasionally had some weeds present, however, not nearly as abundant as the mulch plots. The following weeds were present: Virginia pepperweed (*Lepidium virginicum*),

spanish needles (*Bidens* spp.), Florida purslane (*Richardia scabra*), oldfield toadflax (*Linaria caudensis*), common chickweed (*Stellaria media*), and bristly starbur (*Acanthospermum hispidum*).

Discussion

Of the three types of citrus orchard management practices investigated in this study (irrigation, fertilization, and under the tree ground cover), application of compost material had the most pronounced effects on soil nematodes and nematode community structure. Various genera exhibited different preferences for their microhabitats, suggesting their unique roles and contributions to the soil ecosystem processes. We categorized the different responses to compost applications as three types: I, II, and III.

Type I response. At the generic or family level, the bacterial-feeding nematodes, Rhabditidae and *Cephalobus* showed a typical *r*-selected behavior, with sudden and temporal population increase (within 3-4 weeks) upon mulch additions. This pattern confirms findings other studies of the relationships between organic inputs, microflora, and bacterivorous nematodes. Typically, organic inputs trigger quick increase of bacterial populations followed by a quick increase of some of the bacterivorous nematodes (Ferris et al., 1996; Wasilewska and Bienkowski, 1985; Yeates et al., 1997). As soon as easily decomposable substrates diminish, bacterial and then nematode populations decline usually reaching previous or even lower populations levels. While Rhabditidae showed a similar pattern in organic farming systems in California, *Cephalobus* did not (Ferris et al., 1996). Rhabditidae as strict colonizers ($c-p = 1$) (Bongers, 1990) seem to be affected predominantly by sudden flushes of food resources. Other factors that might be involved in changing the soil microhabitat appear to be less important. This might be the reason why rhabditids are so insensitive to environmental stressors such as pollutants. *Cephalobus*, on the other hand, characterized by Bongers (1990) as not as strong a colonizer ($c-p = 2$) might respond more to a combination of factors (abundance and type of food,

effects of organic material on soil temperature and moisture, natural soil characteristics, etc.). Otherwise, *Cephalobus*, would require some revisions regarding its life traits and thus *c-p* classification. Differences in species of *Cephalobus* or Rhabditidae present or environmental differences between Florida and California may account for these differences in behavior as well.

Aphelenchus and *Aphelenchoides* (fungal-feeding nematodes) also showed a type I response. Interestingly, both genera are not considered strict colonizers (*c-p* = 2), yet in our experiment they behaved like typical *r*-strategists. Results obtained by Ferris et al. (1997) and Yeates et al. (1993b) did not indicate any time-organic input relationship. Wasilewska and Bienkowski (1985), however, observed a delayed numerical response of fungal feeding nematodes to organic matter applications. They suggested that the time-gap between density peaks of bacterial and fungal feeding nematodes reflected slower fungal than bacterial population build-up. Lack of the delay response in our study could result from the type and decomposition state of organic matter. Our well-decomposed mulch probably contained much higher initial bacterial and fungal populations than the dry hay (residues of summer barley) used by Wasilewska and Bienkowski (1985). Since no waiting period for microflora (fungi in particular) build-up was necessary, an immediate response of fungivorous nematodes was observed. Also, the much lower C:N ratio of the municipal waste compost (compared to barley straw) probably facilitated fungal colonization and proliferation.

Aphelenchus, unlike *Aphelenchoides*, was not affected by the first mulch application. It is possible that *Aphelenchoides*, typically a more common fungivore, may have less specialized feeding habits than *Aphelenchus*. If this were the case, the increase of fungus of any type might immediately be reflected in increased nematode density. Unfortunately, the fungal composition of the compost used in these two applications was not determined.

No genera from any other trophic groups could be characterized by the type I response. Typically, bacterial- and fungal-feeding nematodes are associated with decomposition and nutrient mineralization processes (Freckman, 1988; Ingham et al., 1985). Higher densities of type I response nematodes may indicate increased rates of decomposition, possibly resulting in improved mineralization of nitrogen and other nutrients.

Type II response. The bacterial-feeding genera *Acrobeles*, *Acroboloides*, *Eucephalobus*, and *Teratocephalus* were generally suppressed by the presence of compost. Since no temporary numerical response to mulch additions was observed, environmental factors rather than food abundance seem to shape the population dynamics of these bacterivores. *Acrobeles* and *Acroboloides* were also more numerous in tomatoes grown in conventional than in organic systems (Ferris et al., 1997). The suppression by mulch of preferred food types (specific bacteria) is unlikely because a significant time x mulch interaction for *Acroboloides* and *Eucephalobus* was found.

Teratocephalus is a unique bacterial feeder. It is considered a typical *K*-strategist ($c-p = 3$). Presence of compost dropped this nematode to almost undetectable levels. Its density decline in mulch-free plots in the second half of the experiment probably illustrated the unusually dry spring and summer of 1997. McSorley (1997) observed strong positive correlation between rainfall and *Teratocephalus* density in citrus orchards.

In addition, mulch used in our experiment was a municipal solid waste, which contained relatively high concentration of copper. Several recent microcosm studies investigated the effects of pollutants on soil nematodes and concluded that copper concentrations higher than 100 $\mu\text{g/g}$ result in the reduction of nematode abundance (Parmelee et al., 1997; Korthals et al., 1996). On the other hand, Cu is typically bound in non-bioavailable form at high pH, high concentrations of Ca, and high organic matter content (Alva and Graham, 1991).

Besides the above mentioned bacterial feeders, we found *Aporcelaimellus* and *Eudorylaimus* (omnivores) and *Criconemoides* (an herbivore) all exhibiting the type II response. Porazinska et al. (1998) found higher densities of the two omnivorous genera in treatments exposed to higher irrigation intensities. They suggested that higher levels of irrigation were stabilizing the highly variable water and temperature soil environments of sandy soils in Florida. Since the mulch layer contributed to the maintenance of higher soil moisture, lower densities of *Aporcelaimellus* and *Eudorylaimus* were somewhat surprising. We suspect that this response difference was associated with the location of experimental sites. While the irrigation study (Porazinska et al., 1998) was carried out on a site with a slight slope and soils practically never water saturated, this experiment was carried out on lands at a low elevation, occasionally exceeding water field capacity. Therefore, soil water content (here less important) could be overridden by other environmental factors such as, for instance, heavy metal concentrations. Korthals et al. (1996) found omnivorous and predatory nematodes the most sensitive taxa that are negatively affected by copper, nickel, and zinc added to the soil at a concentration of 100 mg/kg.

Criconemoides was suppressed in mulch influenced environments despite availability of host plants (weeds). The suppressive effect of organic materials on ring nematodes has not been reported often (McSorley and Gallaher, 1995; McSorley and Gallaher, 1996). Usually, organic amendments have little effect on ring nematodes.

In general, nematodes showing type II response may indicate the overall status or quality of the soil environment. They probably relate to a combination of food resources and chemical and physical characteristics of their immediate surrounding rather than food availability exclusively.

Type III response. Only three genera were placed in this group: *Plectus* and *Wilsonema* representing bacterivorous nematodes, and *Belonolaimus* representing plant-parasitic nematodes.

In general, presence of mulch created more suitable soil microhabitats hence supporting higher abundances of the type III response nematodes. As in type II response, immediate food availability from mulch additions did not seem to be a key factor affecting population densities. *Plectus* and *Wilsonema* are known as aquatic nematodes favoring wetter habitats. Since soil moisture was higher in mulch treated plots, it is possible that water content was an important factor in providing microhabitats favorable for *Plectus* and *Wilsonema*. *Belonolaimus* also appears to respond more to soil water status than, for instance, to weed abundance. Indeed, weed growth was much greater in compost treatments hence possibly supporting higher populations of *Belonolaimus*. However, the last two sampling dates (September and December 1997) revealed a profound decline in population density of this nematode which may resulted from saturated soils. The rainfall for the autumn of 1997 was unusually high, leaving soil saturated with water for many days. Water-logged soils after two to three days may become oxygen deficient and stimulate sulfate-reducing bacteria to produce sulfur compounds toxic to *Belonolaimus* (Rodriguez-Kabana, 1965).

Again, for some genera, the type III response nematodes, as the type II response nematodes, may reflect more the status of the soil ecosystem rather than availability of food resources.

Trophic groups. The patterns observed at the trophic group level for bacterivores, fungivores, and omnivores resembled patterns of nematodes at the generic level. For example, the density peaks in May 1995 and June 1997 for bacterial feeders were driven by the most abundant rhabditids responding to mulch additions. Higher nematode densities in non-mulch treated plots between the above mentioned peaks manifested the densities of the type II response nematodes (*Acrobeles*, *Acrobeloides*, *Eucephalobus*, and *Teratocephalus*). Although the pattern at the trophic level can identify increased abundance and thus activity of nematodes, in general it does not provide sufficient information about the “players” and possible causes of the observed

trends. Trophic group expresses an average response often neutralizing opposite "roles" of individual species in the soil ecosystem processes. The different responses of nematodes such as Rhabditidae and *Plectus* would not be clear at the trophic group level of resolution. Ferris et al. (1997) provided evidence for different contributions of bacterivorous nematode species to N-mineralization. Different responses of constituent species in the nematode community may indicate their unique and thus critical roles in nutrient and energy flow on a temporary scale. This type of knowledge becomes evident at the lower than trophic group level resolution. However, resident species are usually site specific. If a given niche across sites is filled by different species, trophic groups could express more robust relationships.

The pattern formed by fungivorous nematodes was shaped by *Aphelenchus*. Again, higher abundance of *Aphelenchus* overshadowed the response trend formed by *Aphelenchoides* and other fungal-feeding nematodes. Lack of data on precise feeding preferences of these nematode species and their contributions to nutrient cycling limits our understanding of the roles of these fungivores. Based on findings of Ferris et al. (1997), however, we speculate that fungivores, like bacterivores, differ in their ability to mineralize nitrogen, thus information on the individual genera or even species seems more appropriate.

The resolution of the trophic group for omnivorous nematodes may seem more appropriate in defining soil ecosystem status. Usually relatively low densities of omnivorous nematode genera or species limit observation of clear responses induced by environmental changes at the species or genus level. A natural tendency would be to group them in a functional assemblage, hoping that abundance magnified in this way would reveal a response pattern. As typical *K*-strategists, omnivores, unlike bacterivores, display more or less similar environmental preferences. Here, although genera such as *Ecumenicus* or *Mesodorylaimus* did not indicate any effects from mulch, at the level of a functional group they neither masked nor diluted patterns revealed by *Eudorylaimus* and *Aporcelaimellus*. In addition, lack of temporal effects of mulch

(despite the density increase of microflora and type I response nematodes) on either genus indicates that trophic level provides information similar to genus level. However, because of the limited knowledge on the biology and role of these nematodes in soil processes, we may learn more by monitoring generic or species composition.

Ecological indices. The idea of using ecological indices as indicators of ecosystem quality (e.g. diversity, stability, and resilience) has received increased attention over the last decade. Indices may be useful tools because they not only provide quantitative means to characterize an ecosystem, but also to compare different ecosystems. Previous studies that used ecological indices to express changes in the soil environment induced by various farming tactics include: Ferris et al., 1996; Freckman and Ettema, 1993; McSorley and Frederick, 1996; Porazinska et al., 1998; Yeates and Bird, 1994; and Yeates et al. 1997.

We assume that condensation of taxonomic characterization of the nematode community into a simple ecological index value should provide convenient means for inferences about soil ecosystems (states and processes). In our study, several ecological indices illustrated nematode community changes induced by mulch application. None of the measures, however, could detect any treatment differences due to irrigation or fertilization level (even though a few differences were noticed at the genus and trophic level).

Species richness can indicate ecological stability (Peterson et al., 1998). Generally lower richness in mulch treated plots reflected probably an absence of mononchid and chromadorid genera. Inability to indicate temporary effects of mulch and high variation through time make this index imprecise for describing soil nematode community changes.

Lower trophic diversity in mulch treatments resulted from nematode communities predominated by bacterial and fungal feeders. This inference, however, would be impossible if we had no access to information about the generic and trophic composition.

Simpson's and Shannon-Weaver diversity indices can be used interchangeably despite the scale differences (see Chapter 7). Here, both indices had very similar patterns throughout the entire time of the experiment. They both fluctuated more in mulch treatments, and both responded to temporary changes due to mulch additions (decrease of diversities). These indices seem to be more informative than the other indices just described, although no qualitative inference can be made. Does a decline of a diversity index indicate loss of ecosystem stability and quality only? A fluctuating diversity index can reflect the dynamics of the ecosystems processes such as, for instance, temporary changes of decomposition and nutrient mineralization rate. Again, to be precise about the reasons of the deviations of the diversity index value, a more detailed knowledge of the parties of the community in question is required. The same argument applies to Simpson's dominance. Without prior generic characterization of nematode communities, it would be impossible to know that an increase of dominance after mulch additions was due to higher proportions of rhabditids and aphelenchids.

Fungivorous to bacterivorous nematode ratio can reflect some aspects of the soil environment. It can describe contributions of the above trophic groups to decomposition processes. Similar values of this ratio in mulch and non mulch treated soils during the first two years of the experiment suggest that breakdown of organic matter was proportionately assisted by similar decomposer assemblages of nematodes. The low values of the ratio during this time indicates predominant contribution of bacterial feeders to decomposition and relatively quick turnover of the available organic matter (leaf litter, dropped fruit, decaying weeds, compost). During the third year of the experiment, changes of the soil environment following compost addition resulted in proportional shifts of nematodes associated with decomposition. An increased ratio indicated a switch to fungal pathway and possibly slower rate of organic matter turnover. Higher proportions of fungal-feeding nematodes probably reflected more favorable soil moisture microhabitats for fungi. Although the ratio helps to indicate the parties involved in

the process of decomposition, it failed to recognize the intensity of the process. The information about the effects of mulch on densities of type I nematodes and densities of both trophic groups had become suppressed and thus the ratio inadequately illustrated the differences between the treatments.

Maturity indices seem to offer better prospects for detecting and sufficiently illustrating changes in the soil environment (Bongers, 1990, Yeates, 1994). Unlike diversity measures, maturity indices contain both quantitative and biological-ecological aspects of the individual nematode species comprising a community. However, low values of maturity indices may be associated with either rare *K*-strategists or predominant *r*-selected nematodes. In our experiment, both scenarios could explain the patterns of both maturity indices: *MI* (Bongers, 1990) and *TMI* (Yeates, 1994). Generally lower *MI* and *TMI* in mulch treatments resulted from suppression of *K*-selected omnivores, indicating overall mulch effect on soil environment over a longer time scale. Significant short-lived declines of *MI* and *TMI* values after mulch application illustrated increased abundances and activities of the type I nematodes. Therefore, *MI* and *TMI* may be indicative of temporary intensified decomposition and nutrient mineralization processes. Similar results were recorded by Ferris et al. (1996) for conventional and organic farming systems in California. Lower maturity of organic plots after incorporation of organic material was associated with relatively higher proportions of opportunistic nematodes responding to bacterial blooms.

Although a measure of maturity index may be appealing for monitoring purposes, we suggest they cannot be taken out of the context of agricultural management practices. Soils with high maturity indices could be interpreted as more stabilized and thus more desirable for agricultural endeavors. In our previous work (Porazinska et al., 1998c), we found highest maturity indices in treatments with the most intensive irrigation schemes. Considering water conservation issues and relatively lower productivity and profitability of those treatments, higher

maturity indices would not be advantageous. The same idea applies to this experiment. Despite higher density of the citrus root rot fungus (*Phytophthora*) and higher weed abundance, productivity of mulch-treated trees was always greater, while maturity indices had exactly the opposite pattern. At the current state of knowledge, it would be rather risky to blindly infer about the quality or sustainability of the soil ecosystem from a particular maturity index value. Low maturity indices may more reflect the intensity of soil processes (decomposition, mineralization) instead of simply the level of disturbance or degradation.

Nematode community measures were useful in providing the information about the status and processes of the citrus soil ecosystem. They could reflect ecosystem differences imposed by several farming management practices. A majority of nematode genera and ecological measures could indicate environments influenced by mulch, but not by water or fertilization level. To accurately characterize the status and processes of the soil environment, we suggest the use of nematode community description at the generic (if not species) level. Maturity indices were less precise, yet still useful for quick assessment of the soil status. Without the prior knowledge on the taxonomic characterization of nematode communities, ecological indices (e. g. Shannon-Weaver and Simpson's) were not very informative.

Table 4-1. Analysis of municipal solid waste compost

Element	Composition
pH	7.5
	%
C	29.1
N	1.61
C:N ratio	19.3
	mg g ⁻¹
P	2.9
Ca	24.8
Mg	2.4
K	3.4
Na	ND ¹
	µg g ⁻¹
Zn	423
Cu	165
Mn	210
Fe	8805
Cd	2
Pb	212
Ni	34

 ND¹ = not determined

Table 4-2. Occurrence of significant effects of water (W), mulch (M), and fertilizer (F) treatments, and their interactions on single nematode populations, trophic groups, ecological indices, and other biological and physical characteristics of the soil environment. Each asterisk (*) represents a sampling date (out of 11 total dates) on which a significant (at $P \leq 0.05$) treatment effect or interaction existed.

	Water	Mulch	Fertilizer	W*M	W*F	M*F	W*M*F
<u>Genus or family</u>							
Bacterivores							
Rhabditidae		*****				*	
Monhysterida			**				
<i>Acrobeles</i>		****		*			
<i>Acrobeloides</i>	*	**		**	**	*	
<i>Cephalobus</i>		**					
<i>Eucephalobus</i>		**					
<i>Plectus</i>	*	*****			**		
<i>Zeldia</i>	*	**			*		
<i>Prismatolaimus</i>	*		**		**		
<i>Teratocephalus</i>	**	***	*	*	**	**	
<i>Wilsonema</i>		***	*		*		*
Misc. bacterivores			*				
Fungivores							
<i>Aphelenchus</i>		***					
<i>Aphelenchoides</i>		**		*		*	
Predators							
<i>Prionchulus</i>				**			
Predatory dorylaims	***		**			**	***
Predatory mononchs		*					*
Algivores							
Chromadorida		**				*	
Omnivores							
<i>Eudorylaimus</i>		*	*	*	*		
<i>Aporcelaimellus</i>	*	**			**	*	
Dorylaimidae	**	*	*		**		
Plant associates							
Tylenchidae	**	***	*	*	*		
Herbivores							
<i>Belonolaimus</i>		***	*	*	*		
<i>Hoplotaimus</i>		*					
<i>Criconemoides</i>		**			**		
<u>Trophic groups†</u>							
Bacterivores		**					
Fungivores		***					
Predators	**		**			*	***
Omnivores	*	***	*	*	**		
Total		***			*		
B/T		**		**			
F/T	*	**				*	
B+F/T		*			*	*	
A/T		*				*	
P/T	*		*			*	*
O/T	**	***	*				

TI/T	*	*				
H/T		*				
B+F		****				
<u>Ecological indices†</u>						
Richness		**	*	*		
F/B	*	***		*		*
B+F/H		***				
H'_i		***	*	*		
λ_i		*		*		
H'_g		***				*
λ_g		***				*
$1/\lambda_g$		***			**	*
MI		*****			**	*
TMI		*****		*		
PPI	*	*****	*			**
<u>Other variables</u>						
Moisture	**	***	**		***	
Phytophthora	*	**	**			**
Root weight					*	
Fungi ‡		*			*	*
Bacteria ‡			*			
Yield ‡		***				

† B = bacterivores; F = fungivores; A = algivores; P = predators; O = omnivores; TI = tylenchids; H = herbivores; T = total;

‡ H'_i = Shannon-Weaver trophic diversity; λ_i = Simpson trophic dominance; H'_g = Shannon-Weaver generic diversity; λ_g = Simpson generic dominance; $1/\lambda_g$ = Simpson generic diversity; MI = maturity index; TMI = total maturity index; PPI = plant parasitic index;

§ only three sampling dates for these measures.

Figure 4-1. View of treatments separated by the first canonical into two groups: mulch plots (bars with positive values of the first canonical) and herbicide treatments (bars with negative values of the first canonical). M = mulch, nM = no mulch, rF = reduced amount of fertilizer, sF = standard amount of fertilizer, rW = reduced amount of water, sW = standard amount of water.

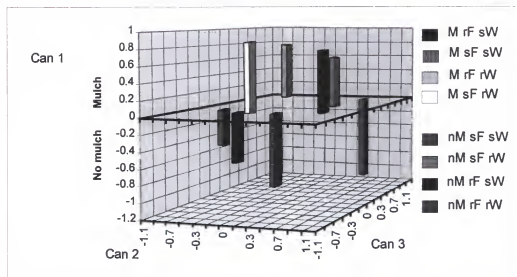
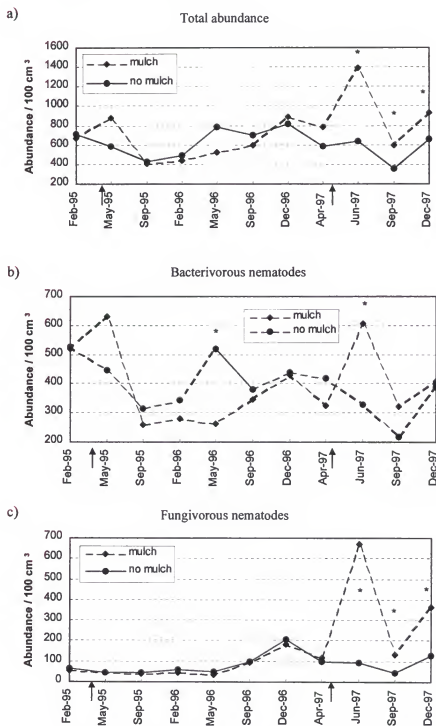
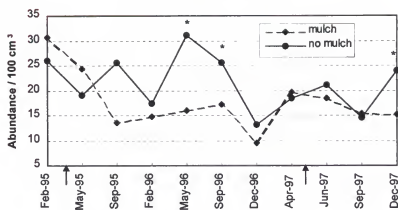


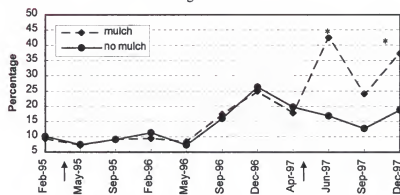
Figure 4-2. Abundance of total nematodes (a), bacterivorous nematodes (b), fungivorous nematodes (c); and omnivorous nematodes per 100 cm³ soil (d). Fungivorous (e) and omnivorous (f) nematodes as a percentage of the total nematode community. Abundance of nematodes associated with decomposition (g) per 100 cm³ soil in mulch- and non mulch- treated plots. Arrows indicate times when mulch was applied. Asterisks indicate significant difference at $P \leq 0.05$.



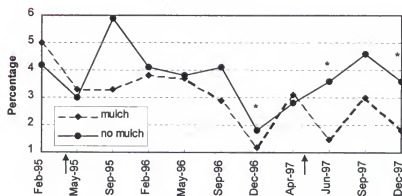
d) Omnivorous nematodes



e) % Fungivores



f) % Omnivores



g)

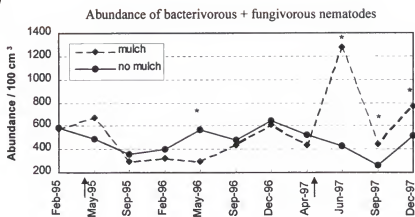
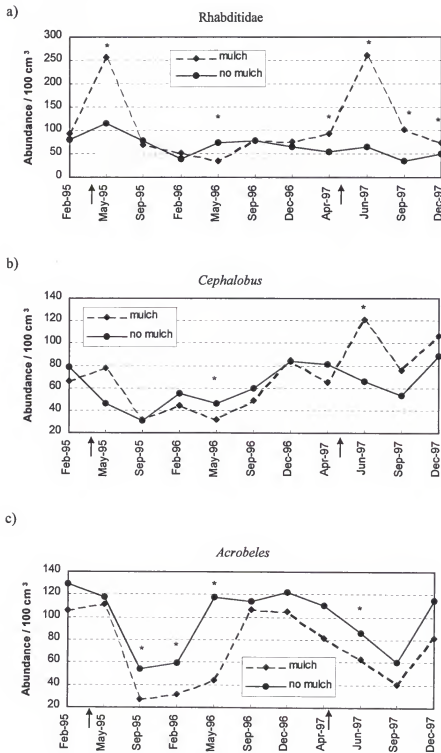
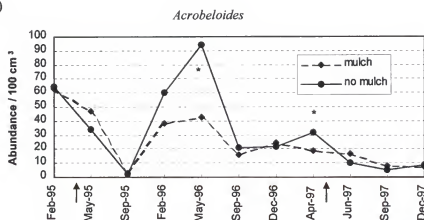


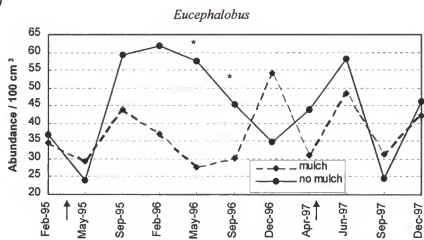
Figure 4-3. Abundance of bacterivorous families or genera: Rhabditidae (a), *Cephalobus* (b), *Acrobes* (c), *Acroboides* (d), *Eucephalobus* (e), *Teratocephalus* (f), *Plectus* (g), and *Wilsonema* (h) per 100 cm³ soil in mulch- and non mulch- treated plots. Arrows indicate times when mulch was applied. Asterisks indicate significant difference at $P \leq 0.05$.



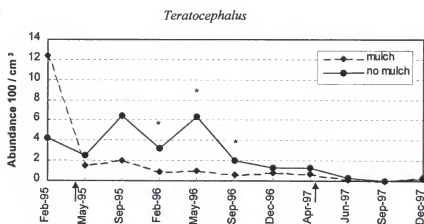
d)



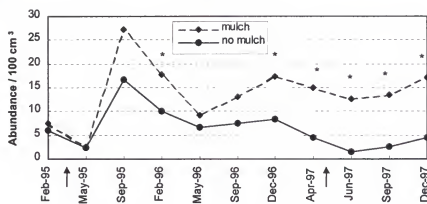
e)



f)



g)

Plectus

h)

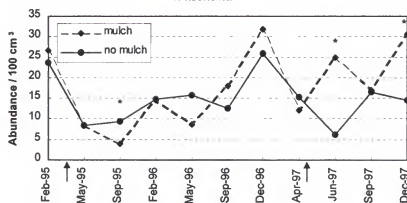
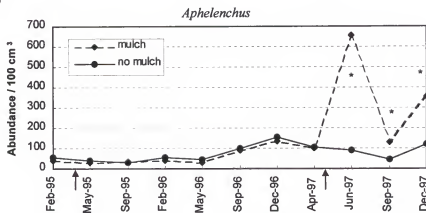
Wilsonema

Figure 4-4. Abundance of fungivorous genera: *Aphelenchus* (a) and *Aphelenchoides* (b) per 100 cm³ soil in mulch- and non mulch- treated plots. Arrows indicate times when mulch was applied. Asterisks (*) indicate significant difference at $P \leq 0.05$.

a)



b)

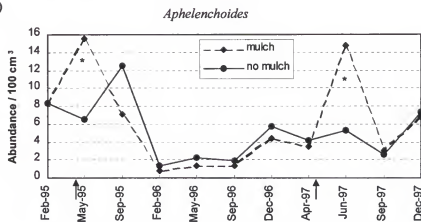
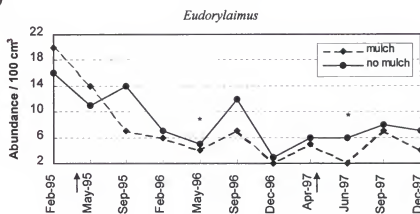


Figure 4-5. Abundance of omnivorous genera: *Eudorylaimus* (a) and *Aporcelaimellus* (b) per 100 cm³ soil in mulch- and non mulch- treated plots. Arrows indicate times when mulch was applied. Asterisks (*) indicate significant difference at $P \leq 0.05$.

a)



b)

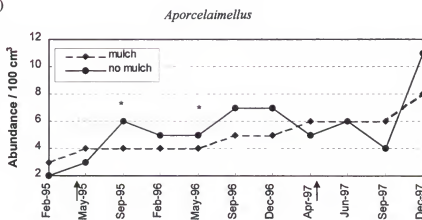


Figure 4-6. Abundance of herbivores: *Belonolaimus* (a), *Cricemoides* (b) and root associates (Tylenchidae) (c) per 100 cm³ soil in mulch- and non mulch- treated plots. Arrows indicate times when mulch was applied. Asterisks (*) indicate significant difference at $P \leq 0.05$.

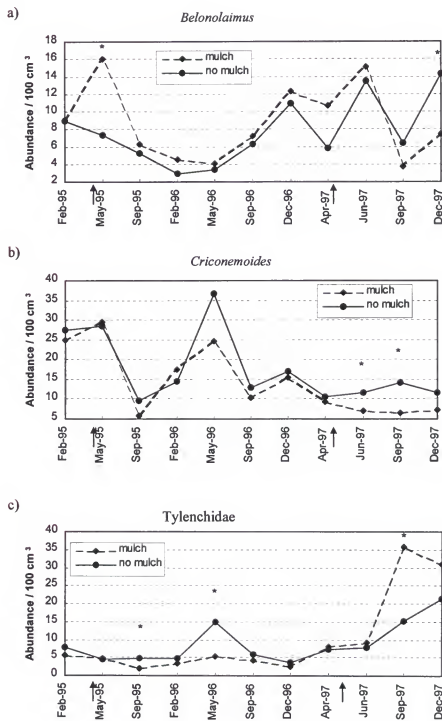
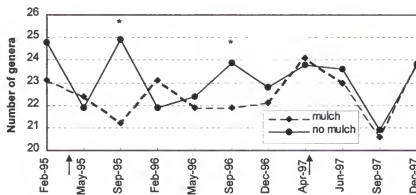
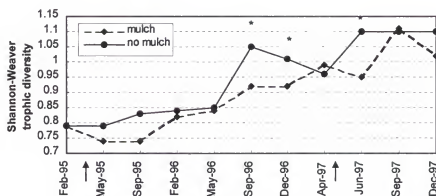


Figure 4-7. Indices of diversity: richness (a), Shannon-Weaver trophic diversity (b), Shannon-Weaver genus diversity (c), Simpson genus diversity (d), and Simpson genus dominance (e) in mulch- and non mulch- treated plots. Arrows indicate times when mulch was applied. Asterisks (*) indicate significant difference at $P \leq 0.05$.

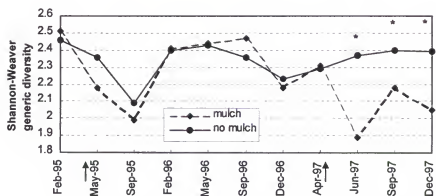
a)



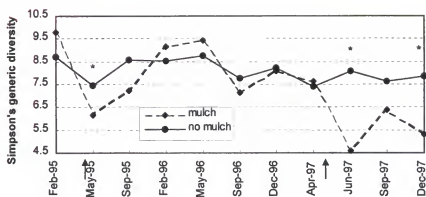
b)



c)



d)



e)

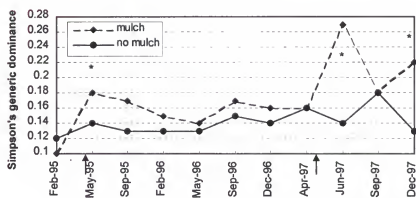
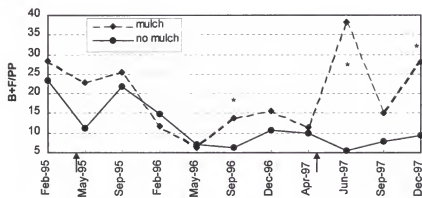


Figure 4-8. Bacterivores + fungivores to plant parasites ratio (a) and fungivores to bacterivores ratio (b) in mulch- and non mulch- treated plots. Arrows indicate times when mulch was applied. Asterisks (*) indicate significant difference at $P \leq 0.05$.

a)



b)

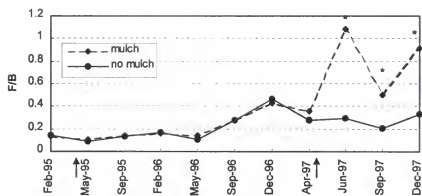
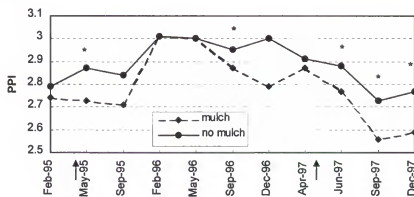
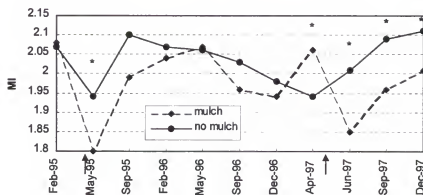


Figure 4-9. Maturity indices: plant-parasitic index (a), maturity index (b), and total maturity index (c) in mulch- and non mulch- treated plots. Arrows (*) indicate times when mulch was applied. Asterisks indicate significant difference at $P \leq 0.05$.

a)



b)



c)

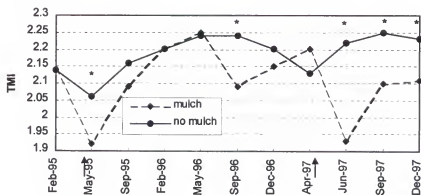
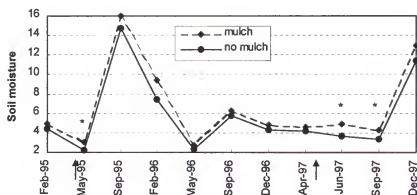
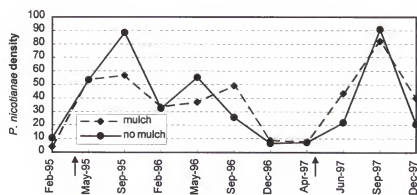


Figure 4-10. Soil moisture (a), *Phytophthora* density (CFU/10 g soil) (b), and primary production (fruit yield) (c) in mulch- and non mulch- treated plots. Arrows indicate times when mulch was applied. Asterisks (*) indicate significant difference at $P \leq 0.05$.

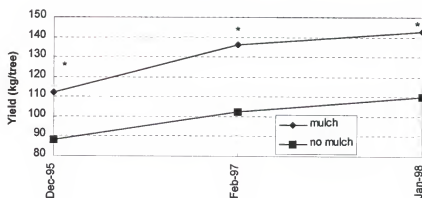
a)



b)



c)



CHAPTER 5

EFFECTS OF VARYING NITROGEN FERTILIZATION ON SOIL NEMATODE AND MICROBIAL COMMUNITIES IN A YOUNG CITRUS ORCHARD

Introduction

Macro- and micro- nutrient availability is crucial for the growth of all plants, including citrus trees. Nutrients removed in the form of fruit yield from a citrus agroecosystem must be replaced to maintain fruit production. Direct application of synthetic fertilizers provides a convenient means for nutrient replacement. Nitrogen and potassium are particularly important elements in Florida citrus orchards, and if these are in short supply, tree growth and fruit production are limited. In general, fruit production and quality improves as more nitrogen and potassium are delivered (Koo, 1988; Obreza and Rouse, 1993; Willis et al., 1990). Application of excessive amounts of fertilizers, however, not only alters fruit quality (increased acid content, peel thickness, greening at harvest, and incidence of creasing and scab) (Jackson et al., 1995) but also contributes to groundwater contamination in Florida (Jackson et al., 1995). Soils of the Central Ridge of Florida are considered highly permeable and well drained. Extremely high sand (96%) and low organic matter (1%) content provide excellent conditions for nutrient leaching. To minimize nutrient losses and groundwater pollution, Florida farmers should apply fertilizers in small but frequent doses.

Mineral fertilization can affect soil biota as well as the chemical and physical soil properties. To detect changes of the soil condition (e. g. nutrient enrichment, loss of soil fertility, accumulation of heavy metals, and loss of diversity), various indicators may be monitored. The soil biota is an excellent prospect for biomonitoring because it integrates effects of treatments on physical and chemical soil properties over a certain period of time. Nematodes

possess several attributes of any prospective bioindicator. They are abundant in virtually all environments, represent a diversity of feeding strategies (algal-, bacterial-, and fungal-feeders, herbivores, omnivores, predators) and life habits (*r-K* strategies) (Freckman, 1988; Yeates et al., 1993), have short life cycles, and relatively well-defined sampling and extraction procedures (McSorley, 1987).

Nematode communities have already been used to describe secondary succession of natural ecosystems or environmental disturbance (Ettema and Bongers, 1993; Freckman and Ettema, 1993; Freckman and Virginia, 1997; de Goede and Dekker, 1993; Wasilewska, 1994; Yeates and Bird, 1994). The idea that changes in the soil environment imposed by agricultural farming practices could be monitored by nematode community measures has been also investigated (Ferris et al., 1996; Freckman and Ettema, 1993; McSorley and Frederick, 1996; Porazinska et al., 1998; Yeates et al. 1997). However, reports on the effects of synthetic fertilizers on the nematode community are scarce and inconsistent (Dmowska and Ilieva, 1995; Sohlenius and Wasilewska, 1984; Todd, 1996; de Goede and Dekker, 1993).

Because Florida soils are N-limited, increased applications of nitrogen might stimulate bacteria as well as nematode bacterial feeders (*r*-strategists) to proliferate. Excessive nitrogen supply, however, might negatively affect nematodes with *K*-oriented life strategies. Therefore, objectives of this study were to: 1) determine if measures of nematode genera and community structure reflected varying amounts of nitrogen fertilizer inputs, and 2) compare information from our study to previous reports on the influence of fertilizer application on the soil nematode community.

Materials and Methods

Site description. The experiment was conducted at the University of Florida Citrus Research and Education Center (CREC) in Lake Alfred, Florida (lat. 28°6'N, long. 81°45'W), and was part of a longer-term study. The soil is characterized as Astatula fine sand with

approximately 1% soil organic matter and pH 6. On 8 September 1993, young citrus trees of 'Hamlin' orange (*Citrus sinensis* (L.) Osbeck) on 'Swingle' citrumelo (*Citrus paradisi* Macf. x *Poncirus trifoliata* (L.) Raf.) rootstock were planted in rows 6 m apart, with 3.7 m between trees in rows. At planting, trees were free *Tylenchulus semipenetrans* Cobb and other nematode pests of citrus. Nematode community data collection began on 31 January 1997.

Treatments and sampling. Trees were fertilized with a synthetic dry soluble fertilizer (N:P:K 17:6:12, Vigoro® MIX 3, Vigoro Corp.) at four different rates equal to 1, 2, 4, and 6 times the lowest application rate during first three years (Table 5-1). During the fourth year of the experiment, the differences between N rates were less drastic and were equal to 1, 1.7, 2.4, and 3.1 times the lowest application. The amount of N differed for all years because rates were adjusted annually to conform to tree growth. To keep the area under the tree canopy free of weeds, the soil surface of the plots was sprayed with glyphosate (N-(phosphonomethyl) glycine, isopropylamine salt). Typically, herbicide was applied 3-4 times per year at about 2- to 3-month intervals starting in late February or early March. Trees were watered with a microsprinkler irrigation system twice a week, except on raining days. To alleviate an increasing pressure from the sting nematode (*Belonolaimus longicaudatus* Rau) on tree growth, oxamyl (formulated as Vydate® L) was applied to all trees at a rate of 2.245 kg ai/ha on 27 April 1997 and 19 June 1997.

The experiment was a randomized complete-block design with five trees per plot (N rate) replicated five times. Data were collected from the fourth year of the experiment which enabled us to observe the longer-term effects of nitrogen treatments on nematode communities. Soil samples were taken under the tree canopy approximately 30 cm away from the tree stem, on 31 January 1997, 19 May 1997, 24 October 1997, and 29 January 1998. Three inner trees (out of five/plot) were sampled by collecting 5 soil cores (2 cm in diameter and 30 cm in length) per tree (15 total soil cores per soil sample). The soil was immediately transported to Gainesville,

Florida, and stored in sealed plastic bags for no more than 15 hours at 10° C. The soil was then mixed and nematodes were extracted from 100-cm³ subsamples by wet sieving followed by centrifugation (Jenkins, 1964). All extracted nematodes were killed with heat, counted, and identified to genus when possible under an inverted microscope. Approximately 5-6 g of soil from every sample was oven-dried for about 24 hour at 60° C to determine soil moisture content. An additional 10-g (dry weight) subsample from each soil sample was used to estimate bacterial and fungal population densities using a dilution plate method (Casida, 1968; Johnson and Curl, 1972). Approximately 25 cm³ of each soil sample were mixed with 50 cm³ of distilled water to measure soil pH.

Response variables. The abundance of each nematode genus and total abundance of nematodes were determined in each sample. All nematode genera were assigned to the following six trophic groups: bacterial feeders, fungal feeders, omnivores, plant parasites, predators, and root associates (Yeates et al., 1993a). The total nematode abundance and the relative abundance of each trophic group for every sample was also recorded. Based on the taxonomic description of the nematode communities, diversity and maturity indices were calculated. Total number of genera was used to determine the effects of different nitrogen treatments on richness. The Shannon-Weaver index (H') of diversity (Shannon and Weaver, 1949) and the Simpson index (λ) of dominance (Simpson, 1949) were used to assess diversity and dominance at both generic and trophic levels. A reciprocal transformation of Simpson's index ($1/\lambda$) (Freckman and Ettema, 1993) was derived to give an additional measure of diversity. Maturity index (MI) (Bongers, 1990) and total maturity index (TMI) (Yeates, 1994) were also calculated. Maturity indices, unlike diversity indices, are more biologically meaningful because they take into consideration biological and ecological characteristics of the constituent nematodes within a nematode community. Nematodes at the family level are assigned colonizer-persister ($c-p$) values ranging from 1 (strict r -strategists) to 5 (strict K -strategists). The $c-p$ scale

relates to nematode life and feeding habits, which presumably can reflect the conditions of the surrounding soil environment (Bongers, 1990). The lower the maturity index value, the more disturbed and unstable soil environment. In general, maturity index is a weighted mean of the individual *c-p* values. While Bongers (1990) differentiated two types of maturity indices: *MI* (includes all feeding types of nematodes except plant parasites) and *PPI* (includes plant parasites only), Yeates (1994) combined these two into one total maturity index (*TMI*). In addition, the fungivore to bacterivore ratio (Freckman and Ettema, 1993) was calculated to provide information about decomposition and nutrient mineralization pathways.

To detect the effects of different fertilization rates on the nematode community structure, microbial populations, and pH data were subjected to two-way (sampling time, fertilizer treatment) analysis of variance and correlation analysis using SAS software (SAS Institute, Cary, NC).

Results

Only a few nematode genera, trophic groups, and nematode community measures responded significantly to fertilizer treatments (Table 5-2). The following nematode genera were present only sporadically and thus listed in the table as miscellaneous: *Chronogaster*, *Teratocephalus* (bacterial feeders); *Ecumenicus*, *Pungentus* (omnivores); *Meloidogyne*, *Hemicycliophora*, *Hoplolaimus*, *Pratylenchus*, *Scutelonema*, *Tylenchulus*, and *Xiphinema* (plant parasites). Rhabditidae were represented by *Cruznema*, *Diploscapter*, and *Rhabditis*. Mononchid predators included *Miconchus*, *Mononchus*, *Mylonchulus*, and *Prionchulus*, and dorylaimid predators included *Nygolaimus*.

Rhabditidae and *Eucephalobus* showed similar patterns throughout all sampling dates. They reached their highest densities at the third nitrogen rate (Jan 98, $P \leq 0.01$) and, based on May 1997 counts, were little affected by the nematicide application (Fig. 5-1a,b). Densities of *Acrobeles* tended to decrease with the highest fertilizer rate. This decreasing trend was

significant in May 1997 (Table 5-3). In May 1997, the abundance of this bacterial feeder was significantly ($P \leq 0.01$) lower than on any other sampling date (Fig. 5-2), but the site was treated with a nematicide in the previous month. Increasing rates of the fertilizers had a positive effect on the fungal feeding *Aphelenchus* ($P \leq 0.01$, Tables 5-2 and 5-3). Negative numerical response to higher fertilizer levels was recorded for miscellaneous dorylaims, but were significant ($P \leq 0.01$) only following the nematicide application (Table 5-3). The lowest densities of *Belonolaimus* were found in the treatments receiving highest amounts of nutrients (Table 5-2 and 5-3, Fig. 5-4). Although the majority of nematode genera were not affected by fertilization level, often their lowest densities occurred in May 1997, which coincided with the nematicide treatment the previous month.

In general, nematode communities were dominated by bacterial feeders (56 to 86%), followed by fungal feeders (6 to 31 %) and plant parasites (1 to 14 %). Omnivores and root associates ranged from 0 to 4%. Predators and algivores were found only sporadically and were absent in most samples. Numbers of bacterivorous nematodes were negatively correlated with an increase in fertilizer rate only in May 1997, and when expressed as the percentage of the total nematode community, only in October 1997 (Table 5-3). Positive significant correlation with fertilizer rate was found both for fungivore densities and fungivores as a proportion of the nematode community in October 1997 and January 1998 (Table 5-3). Levels of omnivorous nematodes declined as fertilizer rate increased, and this trend was significant ($P \leq 0.01$, Table 5-3) in May and October 1997 for absolute densities and May, October 1997, and January 1998 for the relative densities (Fig. 5-5a,b). Within each trophic group, nematodes were least numerous in May 1997.

Total number of nematodes was highest ($P \leq 0.01$) in the highest fertilizer level treatment in January 1998 (data not shown). Lowest densities and an inverse relationship with the treatment level were observed in May 1997. Total number of genera usually declined as the

rate of fertilizer increased (data not shown). In addition, lowest richness was found in May 1997 ($P \leq 0.01$) and it did not recover to its previous levels until January 1998. Shannon-Weaver diversity and Simpson's dominance at the trophic group level showed opposite trends with fertilizer treatments in October 1997 (Table 5-3). While the lowest values of the majority of diversity indices occurred in May 1997, the month soon after nematicide application, the highest value of Shannon-Weaver diversity calculated for trophic groups was observed at this time. Maturity index was the only nematode community measure consistently responding to fertilizer levels (Fig. 5-6). As the rate of nutrient application increased, *MI* value declined (Table 5-3). *MI* was significantly ($P \leq 0.01$) lower in May 1997, but returned to its original levels by January 1998.

While fungal population densities were positively affected by fertilizer rates, bacterial populations remained relatively stable (Tables 5-2, 5-3; Fig. 5-7a,b). The highest fungal and bacterial population densities were both observed in May 1997. While in January 1997 fungivorous nematodes were negatively correlated with fungal population densities ($r^2 = -0.61$, $P \leq 0.01$), in October 1997 the relationship was reversed ($r^2 = 0.62$, $P \leq 0.05$). Fertilizer treatments and time also influenced pH (Table 5-2). Significantly lower pH was observed in May 1997 than on two other sampling dates (5.47 vs. 6.24 and 6.16 for January and October 1997, respectively). An increase of fertilizer rates resulted in decline of pH (6.08 for the lowest nitrogen rates to 5.88 for highest nitrogen levels). This relationship was particularly evident in January and May 1997 with correlation coefficient $r^2 = -0.69$ and $P \leq 0.01$ (Table 5-3).

Discussion

Enrichment of soil nitrogen has been shown to affect nematode communities in different parts of the world to a varying degree. In this study, nematode community structure patterns could indicate different fertilizer rates, but the observed patterns, particularly at the generic level of nematode community characterization, were not stable throughout sampling dates. The

absence of consistent patterns for fertilizer rates was somewhat expected. The highly disturbing effect of nematicide treatment most probably overshadowed the influence of fertilizer levels on nematodes.

Among bacterial feeders, three nematode genera showed different types of responses to nitrogen treatments. The highest reproduction of Rhabditidae and *Eucephalobus* was observed at the highest nitrogen level on two out of four sampling occasions. Additions of nitrogen in the N-limited soils has resulted in an increase of bacterial populations, and thus an increase of bacterial feeding nematode populations (Ferris et al., 1996). However, in our study, bacterial densities fluctuated on the temporal scale, but were relatively stable across different nitrogen rates. Therefore, the availability of food resources could not explain numerical responses of the above-mentioned bacterivorous nematodes. Possibly other abiotic factors such as pH affected these nematodes. In another study, Porazinska et al. (1998a, in press) observed an inverse relationship between pH and densities of *Eucephalobus*. Lack of similar trends in May and October 1997 could possibly be related to treatment of soil with a nematicide.

In general, nitrogen enrichment negatively influenced populations of another bacterivore, *Acrobeles*. Again, we suggest that factors other than food resource availability determined such a response. It is likely that differences of *Acrobeles* densities on a time scale were influenced by the nematicide application in April and June. In three other studies conducted in the vicinity of the experimental site, the numbers of *Acrobeles* were always comparable across sites and sampling time (Porazinska et al., 1998c; Porazinska et al., 1998 a, b, in press).

Fluctuations of the above nematodes and other bacterivorous genera (data not shown) through time seemed to be linked to bacterial population dynamics. Most of the time, relatively high nematode numbers maintained bacterial microflora at its moderate density levels. However, as the nematode populations and thus grazing pressure declined in May 1997, probably

as a result of nematicide soil treatment, bacterial populations reached their peak abundance. The decay of dead nematodes could additionally provide carbon sources for bacterial growth. When the grazing pressure from recovering bacterivorous nematodes increased, bacterial population numbers dropped significantly on later sampling dates. Similar density-dependent patterns were observed by Wasilewska and Bienkowski (1985). During decomposition of organic material, abundance of bacterivorous nematodes followed the bacterial microflora. Also, fluctuations of bacterial-feeding nematodes in organic farming systems coincided with fluctuations of populations of bacteria (Ferris et al., 1996).

Of the fungivorous nematodes, only *Aphelenchus* was affected by nitrogen treatments. *Aphelenchus* abundance correlated positively with higher nitrogen levels. In our study, this response to increased nitrogen levels could be explained by fungal dynamics. Elevated levels of nitrogen reduced soil pH, creating a favorable environment for fungi. More abundant food resources could support larger fungivorous nematode populations. These results are not in agreement with other studies (Sohlenius and Wasilewska, 1984; Sohlenius, 1990) in which an opposite response to nitrogen enrichment was reported. This difference could be attributed to soil type, vegetation, and climatic conditions. The coupling of fungal-feeding nematodes and fungal microflora was also observed through time. Low nematode populations following nematicide treatment allowed for build up of fungal microflora, which dropped as soon as fungivorous nematodes regained their previous abundance. Sohlenius and Wasilewska (1984) reported fungal feeding population densities depressed in response to nitrogen fertilization. They attributed this response to reduced fungal production in fertilized plots. This was not so in our study, perhaps because N-limited Florida soils supplemented with small and frequent nitrogen doses provided good growth conditions for fungi (nitrogen availability and low soil acidity). Fungivores, whether expressed as numbers per 100 cm³ or percentage of the total nematode community, showed patterns of response to nitrogen fertilization similar to *Aphelenchus*. This

was not surprising, since the only other, albeit much less abundant, fungivorous genus was *Aphelenchoides*.

A negative relationship between *Belonolaimus*, a citrus root feeder, and nitrogen level is not well understood. Applications of fertilizers typically increase the incidence of root feeders (Gupta and Yeates, 1997), however, contrasting results also were observed (Sohlenius and Wasilewska, 1984). Since negative correlations with nitrogen rate were observed in the month following nematicide applications, it is possible that better-formed root systems contained higher concentrations of this systemic nematicide. In a previous study, Porazinska et al.(1998a, in press) noticed an inverse relationship between density of *Belonolaimus* and pH. In this study, lower pH associated with treatments of higher nitrogen rates in January and May 1997, might be responsible for the lower densities of *Belonolaimus*. This observation provides an additional stimulus to further investigate this unknown relationship between pH and *Belonolaimus*.

Omnivorous nematodes responded to nitrogen enrichment in the most consistent pattern, declining with increased fertilizer rates. Although this response was consistent with results from other studies (de Goede and Dekker, 1993; Sohlenius and Wasilewska, 1984; Todd, 1996), the reasons are still speculative. Possibly, frequent and high pulses of nitrogen alter soil conditions temporarily (e.g. nitrate concentration, pH, biological control agents), therefore creating fluctuating microhabitats unfavorable for these *K*-strategists.

Typically, higher fertilization rates negatively influenced a number of genera. A particularly detrimental effect of higher nitrogen rates occurred in the month following nematicide application. The most affected genera represented 'persister' life habits and included *Aporcelaimellus*, *Ecumenicus*, *Eudorylaimus*, *Plectus*, *Prismatolaimus*, *Zeldia*, *Wilsonema*, and mononchid and dorylaimid predators. Drastic alteration of the soil environment (combination of fertilizer rates and nematicide) had a prolonged effect on these nematode populations. In fact,

about four months had to pass before detectable population density levels could again be reached.

Diversity indices generally were not useful in detecting soil differences imposed by nitrogen treatments. The Shannon-Weaver trophic diversity index presented an example of misleading information about the status of the environment. Trophic diversity was maximized in the month following nematicide treatment. Individuals surviving this treatment (only about 60% of the previously observed number of genera and 70% of the total abundance) made up a community with more uniformly lower numbers and little dominance, giving relatively high values of the diversity index. Higher diversity is thought to reflect high stability and resilience of the ecosystem (Peterson et al., 1998). However, at this stage, the soil ecosystem of our study was probably the most vulnerable and least resilient to disturbances. A period of about 4 months was required for restoration of this system to its original state in terms of nematode population densities and number of genera.

The best measure adequately illustrating soil condition and processes was maturity index. Not only did it reflect the effect of disturbance by higher nitrogen rates (combination of negative effects on different nematodes within various trophic groups), but also indicated low index values in the time after nematicide application. Among several indices evaluated, this index seems to provide the strongest evidence that biological and ecological information is useful in defining condition of the environment. These results confirm results of our other studies (Porazinska et al., 1998c; Porazinska et al., 1998a, b, in press). In a one-year experiment involving various irrigation rates, maturity index was the most sensitive and dependable measure of water management history, where as higher maturity index values indicated water overuse. Although many ecological indices were able to detect ground-cover effects in a factorial study (irrigation, fertilization, and ground cover; two levels for each factor), maturity indices provided the most accurate and biologically meaningful information. Temporary decline of maturity

indices upon mulch additions reflected positive numerical response of *r*-strategists, indicating intensified processes of decomposition and nutrient mineralization. Lower values of maturity indices in mulch treatments resulted from lower proportions of omnivorous nematodes in the nematode community, illustrating unfavorable physical and/or chemical microhabitats for *K*-strategists. Effects of nitrogen levels in a factorial study, however, were not strong enough to influence measures of the nematode community structure. These various findings indicate that some agricultural practices can outweigh effects of the other ones. Maturity index seems the most robust and useful measure for monitoring purposes; nevertheless, it is important to consider it in the contexts of a particular agroecosystem and of a particular set of farming tactics.

Several nematode genera and ecological indices responded to fertilizer treatments; however, the observed patterns were not stable throughout the time of sampling, possibly due to nematicide application. The most consistent patterns were recorded for omnivorous nematodes at the trophic group level, for which lower abundances and proportions occurred in treatments receiving higher fertilizer rates. Maturity index was the most sensitive measure illustrating soil nitrogen enrichment and disturbance to the nematode community following application of the nematicide. The fungal microflora responded positively to nutrient enrichment, suggesting an N-limited soil environment. Density-dependent patterns between fungal microflora and fungivorous nematodes confirm their interdependent dynamics.

Table 5-1. Description of the experimental treatments.

Year	Treatment	kg N/tree/year	Application frequency [†]	Quantity per application (kg/tree/year)			
				Nov 1993	Mar 1994	Jun 1994	Sept 1994
1	1	0.036	4	0.009	0.009	0.009	0.009
1	2	0.072	4	0.018	0.018	0.018	0.018
1	3	0.144	4	0.036	0.036	0.036	0.036
1	4	0.216	4	0.054	0.054	0.054	0.054
				Feb 1995	Mar 1995	May 1995	Oct 1995
2	1	0.072	4	0.018	0.018	0.018	0.018
2	2	0.144	4	0.036	0.036	0.036	0.036
2	3	0.288	4	0.072	0.072	0.072	0.072
2	4	0.432	4	0.108	0.108	0.108	0.108
				Mar 1996	May 1996	June 1996	Oct 1996
3	1	0.144	4	0.036	0.036	0.036	0.036
3	2	0.288	4	0.072	0.072	0.072	0.072
3	3	0.576	4	0.144	0.144	0.144	0.144
3	4	0.864	4	0.216	0.216	0.216	0.216
				Feb 1997	Mar 1997	May 1997	Oct 1997
4	1	0.274	4	0.069	0.069	0.069	0.069
4	2	0.469	4	0.117	0.117	0.117	0.117
4	3	0.665	4	0.166	0.166	0.166	0.166
4	4	0.860	4	0.215	0.215	0.215	0.215

[†] Application per year.

Table 5-2. Analysis of variance results and overall means for nematode genera, trophic groups, and ecological indices. Analysis of variance results summarized as significance of main effects (date of sampling, fertilizer) and interaction (date*fertilizer). Each mean entry is an average across all sampling dates and treatments (N=80); units are per 100 cm³ soil for nematodes, no units for ratios or indices. B/T = bacterial feeders/total, F/T = fungal feeders/total, O/T = omnivores/total, PP/T = plant parasites/total, P/T = predators/total, RA/T = root associates/total, F/B = fungivorous/bacterivorous nematode ratio, H'_i = Shannon-Weaver trophic diversity, λ_i = Simpson's trophic dominance, H'_g = Shannon-Weaver generic diversity, λ_g = Simpson's generic dominance, $1/\lambda_g$ = Simpson's diversity, MI = maturity index, and TMI = total maturity index.

Nematode measure	Date of sampling	Fertilizer	Date*fertilizer	Mean $\pm$ SD
BACTERIAL FEEDERS				
<i>Acrobeles</i>	P $\leq$ 0.01	P $\leq$ 0.01	P $\leq$ 0.10	71.6 $\pm$ 53.2
<i>Acroboloides</i>	P $\leq$ 0.01	ns	ns	18.4 $\pm$ 14.1
<i>Cephalobus</i>	P $\leq$ 0.01	ns	ns	45.5 $\pm$ 36.5
<i>Eucephalobus</i>	P $\leq$ 0.01	P $\leq$ 0.01	P $\leq$ 0.01	15.4 $\pm$ 24.5
Monhystera	ns	ns	P $\leq$ 0.01	0.5 $\pm$ 0.9
<i>Plectus</i>	P $\leq$ 0.01	ns	ns	0.7 $\pm$ 0.5
<i>Prismatolaimus</i>	P $\leq$ 0.01	ns	ns	16.0 $\pm$ 19.2
Rhabditidae	P $\leq$ 0.01	P $\leq$ 0.01	P $\leq$ 0.01	23.9 $\pm$ 25.2
<i>Wilsonema</i>	P $\leq$ 0.01	ns	ns	3.4 $\pm$ 7.2
<i>Zeldia</i>	P $\leq$ 0.01	ns	ns	16.0 $\pm$ 19.2
Miscellaneous	P $\leq$ 0.01	ns	ns	1.3 $\pm$ 3.9
FUNGAL FEEDERS				
<i>Aphelenchoides</i>	P $\leq$ 0.10	ns	P $\leq$ 0.05	0.8 $\pm$ 1.1
<i>Aphelenchus</i>	P $\leq$ 0.01	P $\leq$ 0.05	ns	27.6 $\pm$ 21.6
OMNIVORES				
<i>Aporcelaimellus</i>	P $\leq$ 0.01	ns	ns	2.6 $\pm$ 3.5
<i>Eudorylaimus</i>	P $\leq$ 0.01	ns	P $\leq$ 0.10	1.8 $\pm$ 2.4
Miscellaneous	P $\leq$ 0.01	ns	ns	1.4 $\pm$ 1.6
PLANT PARASITES				
<i>Belonolaimus</i>	P $\leq$ 0.01	P $\leq$ 0.05	ns	5.5 $\pm$ 5.2
<i>Crictonemoides</i>	ns	ns	ns	4.5 $\pm$ 11.1
Miscellaneous	P $\leq$ 0.01	ns	ns	6.7 $\pm$ 9
PREDATORS				
Mononchids	P $\leq$ 0.01	P $\leq$ 0.10	ns	0.6 $\pm$ 1.4
Dorylaimids	P $\leq$ 0.01	ns	ns	0.2 $\pm$ 0.5
ROOT ASSOCIATES				
Tylenchidae	P $\leq$ 0.01	ns	ns	4.0 $\pm$ 3.8
TROPHIC GROUPS				
Bacterivores	P $\leq$ 0.01	P $\leq$ 0.01	P $\leq$ 0.01	199.2 $\pm$ 141.2
Fungivores	P $\leq$ 0.01	P $\leq$ 0.05	ns	28.4 $\pm$ 21.6
Omnivores	P $\leq$ 0.01	ns	ns	5.8 $\pm$ 5.6
Plant parasites	P $\leq$ 0.01	ns	ns	16.8 $\pm$ 16.7
Predators	P $\leq$ 0.01	ns	ns	0.8 $\pm$ 1.5
B/T	P $\leq$ 0.01	ns	ns	74.1 $\pm$ 10.8

F/T	$P \leq 0.01$	$P \leq 0.05$	ns	13.9 ± 9.8
O/T	$P \leq 0.10$	ns	ns	2.2 ± 1.9
PP/T	$P \leq 0.05$	ns	ns	7.6 ± 6.5
RA/T	$P \leq 0.05$	ns	ns	1.9 ± 1.6

INDICES

Total	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	255.1 ± 162.43
Richness	$P \leq 0.01$	$P \leq 0.10$	ns	15.4 ± 3.8
F/B	$P \leq 0.01$	$P \leq 0.05$	ns	0.21 ± 0.19
H'_t	$P \leq 0.01$	ns	ns	0.79 ± 0.19
λ_t	$P \leq 0.01$	ns	$P \leq 0.10$	0.60 ± 0.12
H'_g	$P \leq 0.01$	ns	ns	2.04 ± 0.17
λ_g	$P \leq 0.01$	ns	ns	0.18 ± 0.04
$1/\lambda_g$	$P \leq 0.01$	ns	ns	5.71 ± 1.06
MI	$P \leq 0.01$	$P \leq 0.01$	ns	1.69 ± 0.25
TMI	$P \leq 0.01$	ns	ns	2.03 ± 0.11
Soil moisture	$P \leq 0.01$	$P \leq 0.01$	ns	4.18 ± 0.61
pH	$P \leq 0.01$	$P \leq 0.01$	ns	5.86 ± 0.76
Bacteria [†]	$P \leq 0.01$	ns	ns	63.09 ± 61.04
Fungi [‡]	$P \leq 0.01$	$P \leq 0.05$	$P \leq 0.10$	93.62 ± 41.07

[†] Bacterial colony forming units $\times 10^3$ per gram of soil (dry weight).

[‡] Fungal colony forming units $\times 10^2$ per gram of soil (dry weight).

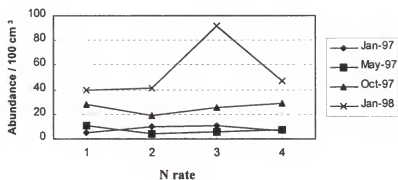
Table 5-3. Correlation coefficients (r) between fertilizer rate and nematode genus, trophic group, or ecological index. B/T = bacterial feeders/total, F/T = fungal feeders/total, O/T = omnivores/total, PP/T = plant parasites/total, F/B = fungivorous/bacterivorous nematode ratio, H'_t = Shannon-Weaver trophic diversity, λ_t = Simpson's trophic dominance, MI = maturity index, and TMI = total maturity index.

Nematode measure	January 1997	May 1997	October 1997	January 1998
BACTERIAL FEEDERS				
<i>Acrobeles</i>	ns	- 0.63**	ns	ns
<i>Acrobeloides</i>	ns	ns	- 0.43*	ns
<i>Cephalobus</i>	ns	- 0.48**	ns	ns
FUNGAL FEEDERS				
<i>Aphelenchus</i>	ns	ns	0.48**	0.44**
OMNIVORES				
Miscellaneous	ns	- 0.53**	ns	ns
PLANT PARASITES				
<i>Belonolaimus</i>	ns	- 0.48**	- 0.44**	ns
ROOT ASSOCIATES				
Tylenchidae	ns	- 0.58**	ns	ns
TROPHIC GROUPS				
Bacterivores	ns	- 0.57**	ns	ns
Fungivores	ns	ns	0.48**	0.43*
Omnivores	ns	- 0.67**	- 0.39*	ns
Plant parasites	ns	ns	ns	- 0.48**
B/T	ns	ns	- 0.63**	ns
F/T	ns	ns	0.54**	0.48**
O/T	ns	- 0.47**	- 0.44**	- 0.45**
PP/T	ns	ns	ns	- 0.45**
INDICES				
Total	ns	- 0.51**	ns	ns
Richness	ns	- 0.58**	ns	ns
F/B	ns	ns	0.53**	0.42**
H'_t	ns	ns	0.50**	ns
λ_t	ns	ns	- 0.62**	ns
MI	ns	- 0.45**	- 0.63**	- 0.58**
TMI	ns	ns	ns	- 0.43*
pH	ns	-0.69**	ns	nd
Soil moisture	ns	ns	0.50**	ns
Fungi	ns	ns	0.48**	0.46**

* $P < 0.05$, ** $P < 0.01$, ns = not significant at $P < 0.05$, nd = no data.

Figures 5-1. Abundances of bacterial feeding nematodes: a) Rhabditidae and b) *Eucephalobus*, respectively, at four different nitrogen rates (1 to 4 with 1 the lowest and 4 the highest N rate)

a)



b)

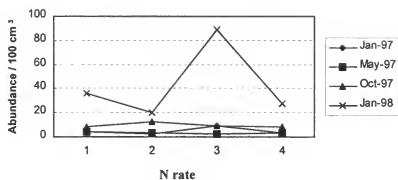


Figure 5-2. Abundance of *Acrobeles* (bacterial feeder) at four different nitrogen rates. (1 to 4 with 1 the lowest and 4 the highest N rate)

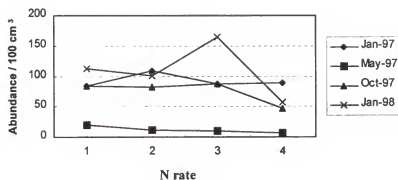


Figure 5-3. Abundance of *Aphelenchus* (fungal feeder) at four different nitrogen rate (1 to 4 with 1 the lowest and 4 the highest N rate).

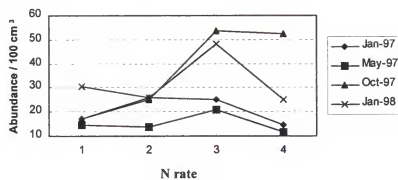
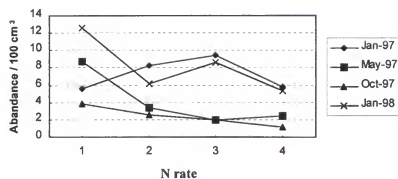
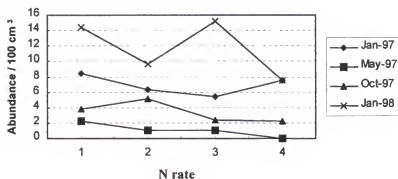


Figure 5-4. Abundance of *Belonolaimus* at four different nitrogen rates (1 to 4 with 1 the lowest and 4 the highest N rate).



Figures 5-5. Abundance (a) and percentage of the total nematode community (b) of omnivorous nematodes at four different nitrogen rates (1 to 4 with 1 the lowest and 4 the highest N rate).

a)



b)

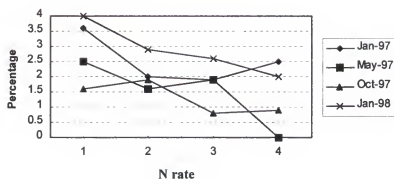
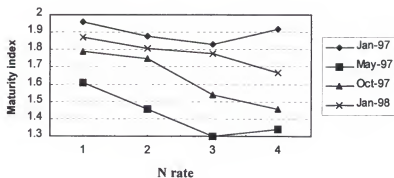
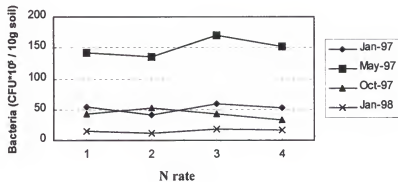


Figure 5-6. Maturity index values in four different nitrogen rates (1 to 4 with 1 the lowest and 4 the highest N rate).

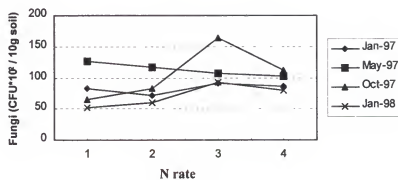


Figures 5-7. Densities of bacterial (a) and fungal (b) colony forming units (CFU) at four different nitrogen rates (1 to 4 with 1 the lowest and 4 the highest N rate).

a)



b)



CHAPTER 6

EFFECT OF DIFFERENT GROUND COVERS ON SOIL NEMATODE COMMUNITIES IN A YOUNG CITRUS ORCHARD

Introduction

Central Florida soils are up to 97% sand with a very small fraction of organic matter (less than 1%). These two key factors result in low soil fertility and low cation exchange capacity (Jackson et al., 1995). Availability of nutrients for plant growth is further limited by nutrient leaching from rainfall and irrigation. Successful crop production systems in Central Florida require considerable nitrogen fertilizer. Nitrogen additions usually improve crop yields, but they also increase the risk of ground water pollution from leaching losses (Syvertsen et al., 1996). Typical citrus management practices involve growing trees in vegetation-free rows with grassy strips between rows of trees. Supplemental irrigation in combination with bare soil within tree rows provides potential conditions for nitrogen leaching through the soil profile and possible contamination of groundwater with nitrates. Recent surveys of Central Florida drinking water wells indicated that 32% of tested wells exceeded safety levels (unpublished data).

To reduce the risk of nitrates in groundwater, standard agricultural management practices need to be modified. Many citrus growers already implement higher N use efficiency techniques (e. g. additions of smaller but more frequent nitrogen rates, more precise placement, more efficient irrigation management). Recently, growing cover crops in citrus orchards has been considered as an alternative management practice to the current practice of maintaining weed free (herbicide-treated) rows between citrus tree. A potentially good candidate cover crop is the rhizoma peanut (*Arachis glabrata* Benth). The rhizoma peanut is a leguminous perennial relatively resistant to drought. The benefits of perennial peanut to citrus production could be

numerous: 1) contribution of nitrogen to the system through N-fixation; 2) lowering soil temperatures through ground shading; 3) reduction of synthetic fertilizer rates; 4) minimization of nitrate leaching; 5) reduction of ground water pollution; 6) possibly an opportunity for establishment of biocontrol agents of citrus pests.

Since plant-parasitic nematodes can limit citrus fruit production (Duncan and Cohn, 1990), much research has focused on methods for minimizing negative effects from nematode pests. Studying entire nematode communities allows for understanding plant-parasitic nematode dynamics in the context of their relations with other nematode feeding types. This more holistic approach provides an excellent opportunity for answering crucial questions about soil ecosystem function (e.g. the relationship between plant parasitic and bacterial feeding nematodes and ecosystem productivity). Abundance of nematodes in all environments and their diversity of feeding habits and life strategies (Freckman, 1988; Yeates et al., 1993a) make them good candidates as bioindicators of the status and processes of an ecosystem (Cairnes et al., 1993). The ability to assess the quality of soils would be of particular importance to farm managers who could modify their farming tactics and thus prevent ecological degradation of agroecosystems and surrounding environment (e. g. soil erosion, loss of soil fertility, ground water pollution, and loss of biodiversity). Several studies investigated whether and to what extent different agricultural practices can be illustrated by nematode communities (Ferris et al., 1996; Freckman and Ettema, 1993; McSorley and Frederick, 1996; Porazinska et al, 1998c; Porazinska et al., b, in press; Todd, 1996; Yeates et al., 1997). The objective of this study was to determine how different ground cover practices affect measures of nematode community structure in a newly-established citrus orchard. We hypothesized that plots under perennial peanut will support more mature (higher proportions of *K*-strategists) and diverse nematode communities than plots continuously treated with a herbicide.

Materials and Methods

The experiment was conducted at the Citrus Research and Education Center (CREC) in Lake Alfred, Florida. The experimental site soil was characterized as Astatula fine sand (96% sand, 3% clay, 1% silt) with pH 6. Perennial peanut (*Arachis glabrata*) rhizomes were planted between rows of four-year-old citrus trees in March 1996. To ensure successful peanut growth, supplemental irrigation was used. Trees were removed in March 1997, and in May 1997, 180 young trees of 'Mid sweet' orange (*Citrus sinensis* L. Osbeck) on 'Rough lemon' (*C. jambhiri* Lush) rootstock were planted into the well-established perennial peanut stand (into area that had previously been between rows). Trees were planted in 9 rows 8 m apart with 3 m between trees in rows. The nursery trees were free of nematode pests. About two months later (July 1997), four ground cover treatments were established: 1) bare soil within and between rows maintained with a use of herbicide; 2) bare soil within rows (herbicide) with successional growth of weeds between rows, occasionally mowed; 3) bare soil within rows with perennial peanut between the rows, occasionally mowed; and 4) peanut within and between the rows. All trees received 189 g of synthetic fertilizer (N:P:K 8:2:8) in July and November 1997 spread evenly in a 1 m diameter area around each tree. During 1998, trees received 220 g of fertilizer in 6 applications starting in March. Trees were watered with a microsprinkler irrigation system usually twice a week. To maintain the ground free of any vegetation in relevant treatments, the herbicide paraquat was applied in June, September, and November 1997, and January and April 1998. Peanut ground cover was mowed in December 1997.

The experiment was a randomized complete-block design with 5 trees per plot replicated six times. Soil samples were collected within 20-30 cm away from the tree stem on 4 August 1997, 8 December 1997, and 11 March 1998. The soil from this 20-30 cm distance around the tree trunk was practically free of weeds or peanut in all herbicide treatments. In trunk to trunk perennial peanut treatment, the 20-30 cm area contained perennial peanut. Each soil sample

consisted of a total of 12 soil cores (2 cm diam x 30 cm depth) collected from the three inner trees (out of five per plot) with four cores per tree. The soil was stored in plastic bags for no more than 15 hours at 10 °C. Prior to nematode extraction, each soil sample was carefully mixed. Nematodes were extracted from 100-cm³ subsamples by wet sieving followed by centrifugation (Jenkins, 1964). All extracted nematodes were killed with heat and identified mostly to genus under an inverted microscope.

The density of each genus/family and the total density across all genera/families were recorded for each sample. Nematode genera were classified to several trophic groups following Yeates et al. (1993a) and included: bacterial feeders, fungal feeders, omnivores, plant parasites, predators, and root associates. Densities (per 100 cm³) and percentages of every trophic group were determined. The taxonomic characterization of nematode communities allowed us to calculate a number of ecological indices. Total number of genera was used to determine the effect of different ground cover types on richness. The Shannon-Weaver diversity index (H') (Shannon and Weaver, 1949) and the Simpson dominance index (λ) (Simpson, 1949) were calculated for both generic and trophic level to assess diversity and dominance of nematode communities. An additional measures of diversity was derived by a reciprocal transformation of Simpson index ($1/\lambda$) (Freckman and Ettema, 1993). Fungivores to bacterivores ratio (Freckman and Ettema, 1993) and fungivores plus bacterivores to plant parasites ratio were calculated to illustrate the treatment differences in decomposition and nutrient mineralization pathways and ecosystem productivity, respectively. Since diversity indices ignore the ecological aspects of the individuals comprising a community, more biologically meaningful maturity indices were calculated: maturity index (MI) and plant-parasitic index (PPI) (Bongers, 1990), and total maturity index (TMI) (Yeates, et al. 1994). Nematode families are assigned colonizer-persister ($c-p$) values (Bongers, 1990). The $c-p$ values relate to biology and ecology of nematodes and range from 1 (strict r -strategists) to 5 (strict K -strategists) and presumably reflect the status of

the soil environment. Maturity indices are calculated as the weighted means of the individual *c-p* values. *MI* includes all nematode feeding types except plant parasites, *PPI* includes only plant parasites, and *TMI* includes all feeding types.

The data were subjected to two-way analysis of variance (sampling date, ground cover treatment) and when necessary to one-way analysis of variance to determine the effects of different ground cover types on the nematode community structure using SAS software (SAS Institute, Cary, NC).

Results and Discussion

From as many as 48 nematode measures (taxonomic, trophic group, and ecological index level), only 15 indicated differences from ground cover treatments (Table 6-1). A vast majority of these variables did not form consistent patterns throughout all sampling dates. Moreover, occasionally repeated significant responses from one sampling date to another were often of opposite nature. In addition, out of those 48 measures, 28 showed a significant ($P \leq 0.01$) effect of sampling time (data not shown).

Total number of 52 different genera were identified (Table 6-2). Some genera were very rare and thus not listed separately in the table. Among bacterial feeders, miscellaneous bacterivores included *Alaimus*, *Chronogaster*, and *Teratocephalus*. The most predominant genus within Rhabditidae was *Rhabditis*, but *Bunonema*, *Cruznema*, and *Diploscapter* were also occasionally present. *Diphtherophora*, *Leptonchus*, *Nothotylenchus*, *Tylencholaimellus*, *Tylencholaimus*, and *Xiphinemella* made up fungal-feeding dorylaims. Miscellaneous omnivorous Dorylaimidae included *Ecumenicus* (the most often encountered genus), *Mesodorylaimus*, and *Pungentus*. Among miscellaneous plant parasites we included *Ecphyadophora*, *Helicotylenchus*, *Pratylenchus*, *Trichodorus*, *Tylenchulus*, and *Xiphinema*. Dorylaimid predators were represented by *Actinolaimus*, *Carcharolaimus*, *Caribinema*,

Nygolaimus, and *Paraxonchium*, while mononchid predators were represented by *Iotonchus*, *Granonchulus*, *Miconchus*, *Mononchus*, *Mylonchulus*, and *Prionchulus*.

Of the many different nematode genera, only *Acrobeloides* (bacterial feeder), *Aphelenchus* (fungal feeder), *Eudorylaimus* (omnivore), mononchid predators, and miscellaneous plant parasites were occasionally affected by the ground cover treatments. *Acrobeloides* was significantly ($P \leq 0.01$) more abundant in both peanut treatments in August 1997, however, in December 1997 the same treatments were characterized by the lowest densities of this nematode genus (Fig. 6-1a). Significantly lower numbers of *Aphelenchus* were recorded in treatments with perennial peanut growing within and between rows in March 1998 than in herbicide trunk-to-trunk plots (Fig. 6-1b). Similar trends were observed during earlier sampling dates, but they were not statistically significant. Trunk-to-trunk peanut treatments had highest abundances of *Eudorylaimus*, however this difference was found significant only in December 1997 (Fig. 6-1c). Predatory mononchids were relatively stable, except in August 1997, when their densities were significantly lower in trunk to trunk peanut treatments (data not shown). In general, miscellaneous plant parasites had lowest densities in treatments involving peanut ground cover, however, this was significant ($P \leq 0.01$) only in March 1998 (Fig. 6-1d).

It is somewhat surprising that not many nematode genera were affected by differences of the ground cover. We expected that quality and diversity of the aboveground vegetation would strongly affect various nematodes from different trophic groups. Treatments with perennial peanut, for instance, might be expected to create environments with lower soil temperature (shading effect) and possibly more stable moisture conditions, leading to nematode communities with higher proportions of *K*-strategists. In an adjacent experimental site, higher irrigation rates resulted in higher abundances of *Aporcelaimellus* and *Eudorylaimus* (Porazinska et al. 1998). High sand content (up to 96%) of Florida soils and high climatic temperatures lead to frequent fluctuations of soil moisture and temperature. Higher irrigation levels had a stabilizing effect of

soil microhabitats allowing an increase in population densities of the nematodes most sensitive to environmental changes. This trend was somewhat confirmed by higher densities of *Eudorylaimus* in peanut treatments.

We expected that the diversity of rhizospheres (citrus, perennial peanut with occasional weeds) would be illustrated by differences in population dynamics of many bacterial feeding nematodes. Other studies showed a differentiating effect of plant species on total numbers of bacterivorous nematodes, indicating that quality and quantity of root exudates play an important role in shaping nematode communities (Griffiths, 1990; Sohlenius, 1990). It was expected that perennial peanut treatments would create soil microhabitats more diverse than herbicide treatments. However, it is possible that a longer time from the establishment of treatments is required to allow all the environmental factors to stabilize and allow distinct microhabitats to develop.

For many nematode genera, the initial sampling date was the most different. While *Acrobeles*, *Acrobeloides*, *Prismatolaimus*, predatory mononchids, *Aporcelaimellus*, *Eudorylaimus*, miscellaneous Dorylaimidae, *Criconeimoides*, and *Meloidogyne* were most abundant in August 1997, *Cephalobus*, *Eucephalobus*, *Plectus*, Rhabditidae, *Aphelenchoides*, *Aphelenchus*, fungal-feeding dorylaims, and miscellaneous plant parasites were least abundant on that date. Many of these more abundant genera (e.g. *Aporcelaimellus*, *Eudorylaimus*, *Prismatolaimus*) are characterized as *K*-strategists, therefore high population numbers in the initial sample may indicate that more than 30 days is necessary for those nematodes to react to soil disturbances (e.g. herbicide application, root death, and tree planting). Other researchers (Ferris et al., 1996; Porazinska et al., 1998b, in press) observed that *r*-selected nematodes required 4-5 weeks to respond numerically to additions of organic materials. Therefore, decomposition of decaying roots of perennial peanut and grasses in trunk to trunk herbicide may not have affected nematode numbers on the first sampling date. In December 1997, reversal in

abundances of the above mentioned nematodes indicated that the disturbance (establishment of treatments) finally affected many nematode genera and its effects were detectable four months following soil disturbance.

In general, no differences among ground cover treatments at the trophic group level were detected in August 1997. Predators were the only exception, with densities significantly ($P \leq 0.05$) lower in peanut trunk-to-trunk treatments (data not shown). Bacterivores and fungivores were more abundant in herbicide trunk-to-trunk treatments in December 1997 and March 1998 (Fig. 6-2a,b). They were least abundant in August 1997, but with time their numbers increased, particularly in herbicide treatments (Fig. 6-2a,b). An increase of bacterivores and fungivores in herbicide treatments may have resulted from decomposition of roots of plants killed by herbicide. Sudden inputs of decomposable organic material usually stimulate proliferation of microflora and nematodes feeding on microflora (Ferris et al., 1996; Griffiths et al., 1993; Porazinska et al., in press; Wasilewska and Bienkowski, 1985; Yeates et al., 1997). These are density-dependent relationships that result from food resource availability (readily decomposable organic material and microflora). In contrast, predators, omnivores, and plant parasite population densities declined with progression of time (Table 6-2). Typically, higher numbers and the percentages of omnivorous nematodes were associated with peanut trunk-to-trunk treatments, but this was significant ($P \leq 0.05$) only in December 1997 and March 1998 when omnivores were expressed as the percentage of the total nematode community (Fig. 6-2c). Higher stability (temperature and food resource) of trunk-to-trunk peanut treatments likely provided favorable environments for omnivorous nematodes. Interestingly, this scenario was observed in this experiment at the genus level for *Eudorylaimus* only in December 1997. Trophic grouping reinforced the effect of trunk-to-trunk peanut cover on strict *K*-strategists, indicating that often the individual generic abundances were too small for treatment differences to be detected. Moreover, lower abundances of predators and omnivores in December 1997 and

March 1998 in comparison to August 1997, suggest that soil disturbances did not have an immediate effect on these *K*-strategists and that a lag-time of more than a month was necessary before those effects could become evident. Typically, soil disturbance (e.g. tillage, liming, fertilization, organic mulching, and burning) is followed by a decline of population numbers of persisters (Sohlenius and Wasilewska, 1984; Todd, 1996; Yeates et al., 1997).

Total number of nematodes was usually not affected by the type of treatment, however, significantly higher densities of nematodes were present in trunk-to-trunk herbicide plots in March 1998 (data not shown). This response was driven by bacterial- and fungal feeders.

At the ecological index level, only two measures were sporadically affected by the type of ground cover treatment. Richness was significantly lower in peanut trunk to trunk treatment in August 1997 (Fig 6-3a). Nematodes absent in this treatment included *Achromadora*, *Monhystera*, *Helicotylenchus*, *Xiphinema*, and fungal-feeding dorylaims. Higher richness in herbicide plots probably reflects an increased microfloral activity due to disturbance and root decomposition (bacterial and fungal feeding nematodes). There were no differences among treatments on later sampling occasions. Across all sampling dates, the total number of genera reached its lowest values in March 1998. An increase of richness, rather than a decrease, would be expected with succession time. Decrease in richness over time involved mostly *K*-strategists (dorylaims and mononchids), which suggests that the initial disturbance (set up of treatments) had a prolonged effect on these nematodes and a scale of years rather than months may be required to observe their recovery.

Trophic diversity was significantly higher in trunk-to-trunk peanut treatment in December 1997. During other sampling dates, no difference between treatments was observed. In general, trophic diversity declined with time (data not shown).

Occasional treatment differences were observed in the maturity indices *TMI* and *PPI*, however, observed patterns were short-lived. *TMI* was significantly higher in August 1997 than

on any other sampling dates (Fig. 6-3b). This again supports the idea that nematode communities may not respond immediately to soil changes. However, introduced disturbance had a prolonged effect on nematode communities. A time period of seven months was not enough for nematodes to restore the *TMI* to values observed shortly after treatment establishment. Seasonal fluctuations of nematode genera also might have affected the *TMI* masking the effect of treatments.

In conclusion, different ground cover treatments had very little effect on nematode communities in this very young citrus orchard. The differences among treatments were present but sporadic and inconsistent. Nematode communities often reflected the initial disturbance due to treatment establishment. Since the recovery of nematode communities to their original form did not occur after seven months, we suggest that more time is required to observe treatment effects. Therefore, nematode communities may not be good indicators of soil differences influenced by ground cover types in the first year following establishment of ground cover treatments.

Table 6-1. Analysis of variance results indicating significance of ground cover treatment effect during three consecutive sampling dates for nematode taxon, trophic group, and ecological index.

Nematode measure	August 1997	December 1997	March 1998
Nematode taxon			
<i>Acrobes</i>	$P \leq 0.1$	$P \leq 0.05$	ns
<i>Acrobeloides</i>	ns	ns	ns
<i>Aphelenchoides</i>	ns	ns	ns
<i>Aphelenchus</i>	ns	ns	$P \leq 0.10$
<i>Aporcelaimellus</i>	ns	ns	ns
<i>Belonolaimus</i>	ns	ns	ns
<i>Cephalobus</i>	ns	ns	ns
Chromadorida	ns	ns	ns
<i>Criconemoides</i>	ns	ns	ns
Dorylaimidae	ns	ns	$P \leq 0.10$
Dorylaimid predators	ns	ns	ns
<i>Eucephalobus</i>	ns	ns	ns
<i>Eudorylaimus</i>	ns	$P \leq 0.05$	ns
Fungal-feeding Dorylaims	ns	ns	ns
<i>Hoplolaimus</i>	ns	ns	ns
Miscellaneous bacterivores	ns	ns	ns
Miscellaneous plant parasites	ns	ns	$P \leq 0.01$
<i>Meloidogyne</i>	ns	ns	ns
Monhysterida	ns	ns	ns
Mononchid predators	$P \leq 0.05$	ns	ns
<i>Plectus</i>	ns	ns	ns
<i>Prismatolaimus</i>	ns	ns	ns
Rhabditidae	ns	ns	ns
Tylenchidae	ns	ns	ns
<i>Wilsonema</i>	ns	ns	ns
<i>Zeldia</i>	ns	ns	ns
Trophic group			
Bacterivores	ns	$P \leq 0.05$	$P \leq 0.01$
Fungivores	ns	ns	$P \leq 0.10$
Omnivores	ns	ns	ns
Predators	$P \leq 0.05$	ns	ns
Plant parasites	ns	ns	ns
B/T	ns	ns	ns
F/T	ns	ns	$P \leq 0.05$
O/T	ns	$P \leq 0.10$	$P \leq 0.05$
Pr/T	ns	ns	ns
PP/T	ns	ns	ns
Indices			
Totals	ns	ns	$P \leq 0.05$
Richness	$P \leq 0.01$	ns	ns
F/B	ns	ns	ns
F+B/PP	ns	ns	ns

Trophic diversity (H')	ns	$P \leq 0.10$	ns
Trophic dominance (λ)	ns	ns	ns
Generic diversity (H')	ns	ns	ns
Generic dominance (λ)	ns	ns	ns
Generic diversity ($1/\lambda$)	ns	ns	ns
<i>TMI</i>	ns	$P \leq 0.10$	ns
<i>MI</i>	ns	ns	ns
<i>PPI</i>	ns	ns	ns

B/T = bacterial feeders/total, F/T = fungal feeders/total, O/T = omnivores/total, PP/T = plant parasites/total, Pr/T = predators/total, F/B = fungivorous/bacterivorous nematode ratio, H' = Shannon-Weaver diversity index, λ = Simpson's dominance, $1/\lambda_g$ = Simpson's diversity, *TMI* = total maturity, *MI* = maturity index, and *PPI* = plant parasitic index

Table 6-2. Means $\pm$ standard deviations for nematode taxa, trophic groups, and ecological indices. Each entry is a mean $\pm$ standard deviation across all treatments per 100 cm³ soil (n = 24).

Nematode measure	August 1997	December 1997	March 1998
Bacterivores			
<i>Acrobeles</i>	67 $\pm$ 61 ^a	36 $\pm$ 25	57 $\pm$ 66
<i>Acroboloides</i>	27 $\pm$ 13	12 $\pm$ 14	21 $\pm$ 12
<i>Cephalobus</i>	73 $\pm$ 24	114 $\pm$ 42	98 $\pm$ 45
Chromadorida	1 $\pm$ 1	1 $\pm$ 1	1 $\pm$
<i>Eucephalobus</i>	9 $\pm$ 6	25 $\pm$ 18	29 $\pm$ 25
Miscellaneous bacterivores	1 $\pm$ 2	1 $\pm$ 2	0 $\pm$ 0
Monhysterida	4 $\pm$ 6	4 $\pm$ 4	3 $\pm$ 3
<i>Plectus</i>	9 $\pm$ 7	16 $\pm$ 9	14 $\pm$ 7
<i>Prismatolaimus</i>	32 $\pm$ 29	16 $\pm$ 8	17 $\pm$ 12
Rhabditidae	35 $\pm$ 16	160 $\pm$ 473	66 $\pm$ 22
<i>Wilsonema</i>	24 $\pm$ 20	24 $\pm$ 17	10 $\pm$ 6
<i>Zeldia</i>	22 $\pm$ 16	20 $\pm$ 26	20 $\pm$ 16
Fungivores			
<i>Aphelenchoides</i>	4 $\pm$ 4	9 $\pm$ 6	9 $\pm$ 10
<i>Aphelenchus</i>	27 $\pm$ 11	40 $\pm$ 25	38 $\pm$ 24
Fungal-feeding Dorylaimida	0 $\pm$ 1	2 $\pm$ 2	1 $\pm$ 2
Omnivores			
<i>Aporcelaimellus</i>	14 $\pm$ 9	5 $\pm$ 3	4 $\pm$ 7
<i>Eudorylaimus</i>	6 $\pm$ 6	5 $\pm$ 3	4 $\pm$ 7
Miscellaneous Dorylaimidae	17 $\pm$ 13	5 $\pm$ 4	5 $\pm$ 4
Plant parasites			
<i>Belonolaimus</i>	1 $\pm$ 1	1 $\pm$ 1	1 $\pm$ 2
<i>Criconemoides</i>	68 $\pm$ 77	50 $\pm$ 48	56 $\pm$ 67
<i>Hoplolaimus</i>	14 $\pm$ 9	16 $\pm$ 9	13 $\pm$ 7
<i>Meloidogyne</i>	39 $\pm$ 20	35 $\pm$ 21	28 $\pm$ 16
Miscellaneous plant parasites	5 $\pm$ 12	7 $\pm$ 12	8 $\pm$ 10
Predators			
Dorylaimid predators	1 $\pm$ 1	1 $\pm$ 2	1 $\pm$ 2
Mononchid predators	13 $\pm$ 12	4 $\pm$ 4	2 $\pm$ 2
Root associates			
Tylenchidae	30 $\pm$ 19	42 $\pm$ 21	27 $\pm$ 15
Trophic group			
Bacterivores	303 $\pm$ 113	334 $\pm$ 92	336 $\pm$ 98
Fungivores	32 $\pm$ 13	51 $\pm$ 29	49 $\pm$ 31
Omnivores	37 $\pm$ 21	16 $\pm$ 6	14 $\pm$ 11
Predators	15 $\pm$ 12	5 $\pm$ 4	3 $\pm$ 3
Plant parasites	127 $\pm$ 79	110 $\pm$ 79	106 $\pm$ 72
B/T	55.5 $\pm$ 9.7 ^b	59.6 $\pm$ 8.1	63.7 $\pm$ 9.1
F/T	6.0 $\pm$ 2.4	9.2 $\pm$ 4.0	8.5 $\pm$ 3.2
O/T	7.0 $\pm$ 3.6	3.0 $\pm$ 1.2	2.8 $\pm$ 2.1
Pr/T	2.9 $\pm$ 2.3	1.0 $\pm$ 0.9	0.6 $\pm$ 0.6
PP/T	23.1 $\pm$ 10.7	19.6 $\pm$ 8.3	19.3 $\pm$ 8.0

Ecological index

Totals	544 ± 161	560 ± 136	536 ± 172
Richness	26 ± 2	26 ± 2	24 ± 2
<i>F/B</i>	0.11 ± 0.04	0.16 ± 0.07	0.14 ± 0.07
<i>F+B/PP</i>	3.66 ± 2.57	4.56 ± 2.75	4.82 ± 3.14
Trophic diversity (H')	1.23 ± 0.12	1.16 ± 0.17	1.06 ± 0.13
Trophic dominance (λ)	0.40 ± 0.07	0.42 ± 0.07	0.47 ± 0.08
Generic diversity (H')	2.60 ± 0.14	2.53 ± 0.13	2.52 ± 0.16
Generic dominance (λ)	0.11 ± 0.03	0.11 ± 0.02	0.12 ± 0.03
Generic diversity ($1/\lambda$)	9.92 ± 2.35	8.94 ± 1.68	9.10 ± 1.78
<i>TMI</i>	2.49 ± 0.17	2.23 ± 0.12	2.19 ± 0.10
<i>MI</i>	2.35 ± 0.19	2.02 ± 0.11	1.99 ± 0.11
<i>PPI</i>	2.79 ± 0.04	2.69 ± 0.03	2.77 ± 0.03

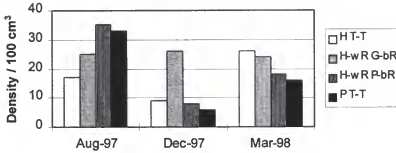
^a nematode taxa expressed as numbers / 100 cm³

^b ratios and indices are dimensionless, except totals (numbers / 100 cm³) and richness (number of genera / 100 cm³).

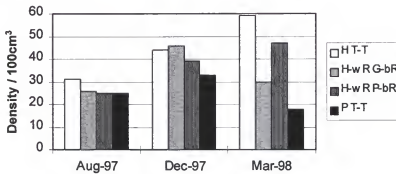
B/T = bacterial feeders/total, F/T = fungal feeders/total, O/T = omnivores/total, PP/T = plant parasites/total, Pr/T = predators/total, *F/B* = fungivorous/bacterivorous nematode ratio, H' = Shannon-Weaver diversity index, λ = Simpson's dominance, $1/\lambda_g$ = Simpson's diversity, *TMI* = total maturity, *MI* = maturity index, and *PPI* = plant parasitic index.

Figure 6-1. Abundance of *Acrobeloides* (bacterial feeder) (a), *Aphelenchus* (fungal feeder) (b), *Eudorylaimus* (omnivore) (c), and miscellaneous plant parasites (d) per 100 cm³ under four ground cover types. H T-T: herbicide trunk-to-trunk (within and between tree rows); H-wR G-bR: herbicide within tree rows with successional weeds between tree rows; H-wR P-bR: herbicide within tree rows with peanut between tree rows; P T-T: peanut trunk-to-trunk.

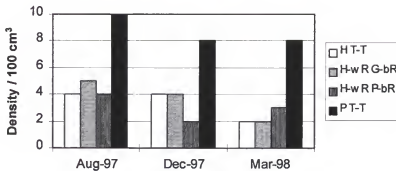
a)



b)



c)



d)

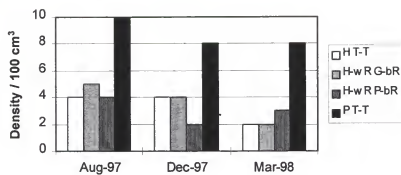
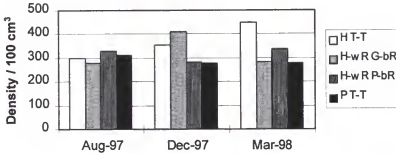
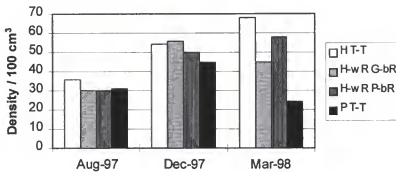


Figure 6-2. Abundance of bacterivorous (a) and fungivorous (b) nematodes and percentage of omnivorous nematodes (c) per 100 cm³ under four ground cover types. H T-T: herbicide trunk-to-trunk (within and between tree rows); H-wR G-bR: herbicide within tree rows with successional weeds between tree rows; H-wR P-bR: herbicide within tree rows with peanut between tree rows; P T-T: peanut trunk to trunk.

a)



b)



c)

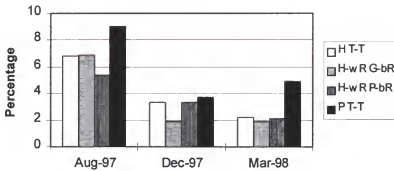
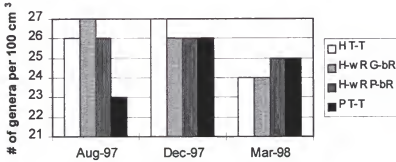
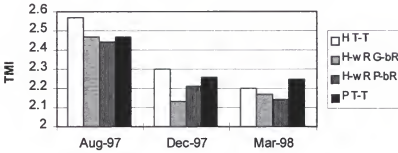


Figure 6-3. Richness (number of genera) and total maturity index (*TMI*) under four ground cover types. H T-T: herbicide trunk-to-trunk (within and between tree rows); H-wR G-bR: herbicide within tree rows with successional weeds between tree rows; H-wR P-bR: herbicide within tree rows with peanut between tree rows; P T-T: peanut trunk-to-trunk.

a)



b)



CHAPTER 7

RELATIONSHIP BETWEEN THE SHANNON-WEAVER DIVERSITY INDEX AND A RECIPROCAL TRANSFORMATION OF THE SIMPSON DOMINANCE INDEX.

Introduction

The multidimensional nature of biological diversity has stimulated scientific thought for decades and resulted in formulation of many ecological theories. Relationships between diversity and ecosystem stability, ecosystem resilience, ecological redundancy, and ecological function are particularly relevant in conservation and sustainable development efforts (De Leo and Levin, 1997; Peterson et al., 1998; Walker, 1991). Some of the crucial questions not yet fully answered include problems of measurement methods and scale (temporal and spatial). A variety of indices are available for the measurement of diversity (Ludwig and Reynolds, 1988). At the community level, two of the most commonly used diversity measures are:

Shannon-Weaver diversity index (Shannon and Weaver, 1949)

$$H = - \sum p_i \log_e p_i \quad (1)$$

where p_i is the proportion of the i th species in the sample, and Simpson's dominance index (Simpson, 1949)

$$\lambda_i = \sum (p_i)^2 \quad (2)$$

The reciprocal transformation of the Simpson's dominance offers an alternative method to measure diversity:

$$1/\lambda = 1/\sum (p_i)^2 \quad (3)$$

The above-mentioned diversity indices are often used in nematode community studies, where the generic or even trophic level is often substituted for the species levels in computing diversity,

due to the difficulty in recognizing undescribed species (Wasilewska, 1984; Freckman and Ettema, 1993).

Materials and Methods

During the last four years, we conducted one three-year-long and three one-year-long experiments to investigate the effects of various management practices (irrigation, fertilization, ground covers, organic mulching) on the structure of nematode communities. For the purpose of assessment and monitoring of soil status and processes, we wanted to determine which of the indices commonly used for measuring nematode diversity could most adequately illustrate the differences in soil environments influenced by various management tactics. The details of the rationales, specific objectives, and experimental designs for each project can be found elsewhere (Porazinska et al., 1998c; Porazinska et al., 1998a, b, in press; Chapter 5 and 6).

Results and Discussion

While analyzing nematode community data from the three-year-long experiment, it was evident that the Shannon-Weaver diversity index and the modified Simpson diversity index were similarly sensitive in detecting differences of soil environments and nematode diversity, both with and without presence of organic mulch (Porazinska et al., 1998b, in press). Although representing different numerical ranges, both indices formed nearly parallel patterns on a temporal scale. Similar results were observed for the data obtained from the three one-year experiments. These observations led to an investigation of the relationship between the above mentioned diversity indices.

For the data set from each experiment, the relationships between H and $1/\lambda$ and between H and $\ln(1/\lambda)$ were derived using Excel software (Microsoft Corporation, Roselle, IL). Similar analyses were performed on the entire set of data pooled from all four experiments.

Regression analyses revealed a highly significant ($P \leq 0.01$) relationship which could predict values of the Shannon-Weaver index from values of Simpson diversity index (Figures 7-

1 and 7-2; Table 7-1). In general, logarithmic models provided slightly better fit to the relationship than linear models ($r^2 = 0.64-0.92$ and $r^2 = 0.64-0.89$, respectively). For values of the Simpson diversity index lower than 4.0, a log effect was observed, therefore logarithmic models were preferred. In addition, the regression coefficients for data from individual experiments and for the data pooled together were of similar value (Table 7-1), suggesting that a similar predictive relationship might apply in all cases.

In conclusion, both the Shannon-Weaver and Simpson indices provide measures of community diversity. Both indices appear to provide similar information in describing patterns in the diversity of taxa. Personal preferences determine which diversity indices are calculated and reported. For quick reference, a regression equation predicting Shannon-Weaver values from the known Simpson diversity values might be useful, particularly for studies involving metaanalysis. Unavailability of large data sets for such relationships limits the possibility of revealing and describing existing patterns or generalizations.

Table 7-1. Coefficients of regression equations of the Shannon Weaver index (H) on the reciprocal of the Simpson's dominance ($1/\lambda$) or $\ln(1/\lambda)$ for nematode community data sets from four different experiments.

Nematode Data Set	Linear Model				Logarithmic Model		
	N	β_0^a	β_1^b	r^2	β_0	β_1	r^2
Irrigation rates ^c	96	1.469	0.109	0.893	0.629	0.830	0.918
Fertilization ^d	80	1.311	0.127	0.643	0.823	0.705	0.642
Ground-cover ^e	72	1.926	0.067	0.849	1.172	0.623	0.876
Factorial ^f	352	1.320	0.129	0.767	0.797	0.769	0.883
Overall ^g	600	1.357	0.124	0.793	0.781	0.771	0.870

^a β_0 = intercept;

^b β_1 = slope;

^c Experiment involved monitoring nematode communities under 6 irrigation rates in a six-year-old citrus orchard (Porazinska et al., 1998c). Data were collected four times (seasons) over one year and pooled across dates for the regression analysis

^d Experiment involved monitoring nematode communities under 4 nitrogen fertilizer rates in a four-year-old citrus orchard (Chapter 5). Data were collected four times (seasons) over one year, and pooled across dates for the regression analysis.

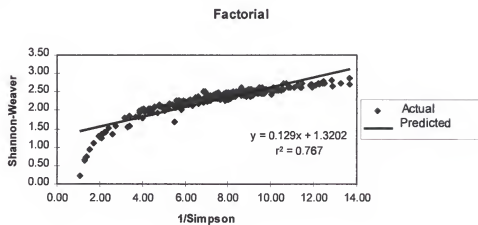
^e Experiment involved monitoring nematode communities under 4 different ground-cover types (Chapter 6). Data were collected four times (seasons) over one year and pooled across dates for the regression analysis.

^f Experiment involved monitoring nematode communities under a combination of three factors (irrigation, fertilization, and ground cover) at two levels each (Porazinska et al., 1998b, in press). Data were collected 11 times over three years and pooled across dates for the regression analysis.

^g Overall data pooled across all experiments and dates.

Figure 7-1. Linear (a) and logarithmic (b) regression models relating Shannon-Weaver index to reciprocal of Simpson index for soil nematode community data in a factorial experiment (N=352).

a)



b)

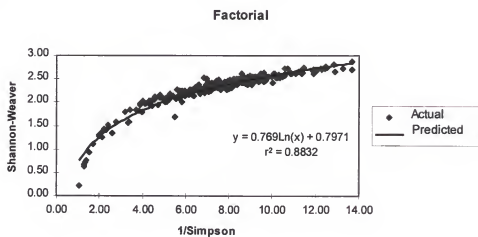
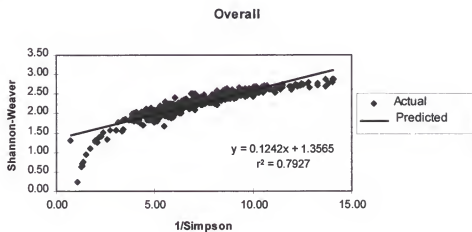
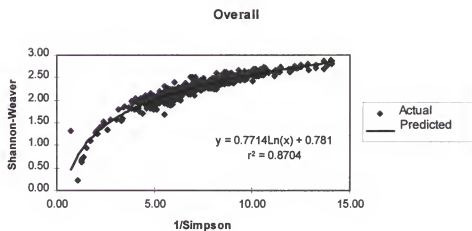


Figure 7-2. Linear (a) and logarithmic (b) regression models relating Shannon-Weaver index to reciprocal of Simpson index for soil nematode community data pooled over four experiments (N=600).

a)



b)



CHAPTER 8 RESEARCH SUMMARY AND CONCLUSIONS

Nematode community measures were often useful for characterizing the status and processes of the citrus soil ecosystem. They reflected ecosystem differences imposed by several farming practices. In single-factor experiments, well-irrigated citrus soils in Florida promoted favorable soil conditions for the nematode trophic group (omnivores) most sensitive to environmental disturbance. More stable soil microhabitats resulting from higher and regular irrigation rates were indicated by higher abundances of *Aporcelaimellus* and *Eudorylaimus*, higher proportions of omnivorous nematodes, and higher values of maturity indices. However, conservation of natural resources, including water, is a priority in sustainable agriculture. Therefore, the inputs required to stabilize soil moisture conditions in this environment conflict with system sustainability, and were reflected by artificially high maturity indices.

Several nematode genera and ecological indices responded to fertilizer treatments. The observed trends, however, were not persistent throughout all sampling occasions. The most consistent patterns were recorded for omnivorous nematodes and maturity index. Lower proportions of omnivores and lower values of maturity index occurred in treatments receiving higher fertilizer rates. Higher nitrogen rates provided stronger pulses of “disturbance” and resulted in more temporarily variable soil micro-environments.

Ground cover treatments had little effect on nematode communities. Some differences among treatments were present but were sporadic and inconsistent. Since the ground cover experiment was in its first year, nematode communities may require more time to reflect treatment effects. However, nematode communities in plots with perennial peanut grown within

and between rows were relatively more mature (higher numbers of omnivores and higher total maturity index) than those in plots with bare soil.

In a multifactorial experiment, the majority of nematode genera and ecological measures of nematode community structure responded to mulch only and could therefore serve as indicators of environments influenced by mulch, but not by water or fertilization level. Characterization of the status and processes of the soil habitat was most accurate using nematode community descriptors at the generic/family level. For instance, *Rhabditidae* might be indicative of intensity of decomposition and nutrient mineralization, and *Aporcelaimellus*, of the overall status of the soil environment (e.g., stability, temperature, and chemistry). Characterization of the soil status by trophic groups often obscured information about the ecosystem. This applied particularly to bacterial feeding nematodes, whose genera often showed totally opposite response patterns to mulch additions. Grouping nematodes of different life strategies (both *r* and *K* common for bacterivores) can result in lack of evident effects observable at the generic/family level of resolution. In contrast, omnivorous nematodes are exclusively *K*-strategists. Therefore grouping of the not too abundant omnivore genera may actually reinforce often weak patterns at the generic level. While maturity indices were still useful for quick assessment of the soil status (but less precise than generic/family characterization), diversity indices (e. g. Shannon-Weaver, Simpson's) were not, because they do not incorporate behavioral information.

Under conditions in which only one agricultural practice was altered (e.g., irrigation rates), nematode communities reflected treatment differences. Nematode communities responded to combinations of farming tactics (multifactorial experiment) by reflecting a dominating factor, indicating that various agricultural management techniques influence nematode communities to different degrees. Time is an additional important factor affecting response of nematode communities to treatment. The effect of treatments were best illustrated in experiments influenced by a particular set of agricultural practices over a longer time period

(irrigation and multifactorial experiments), while in short-term experiments (ground cover experiment in particular), the influence of treatments was not well-reflected by nematode community structure measures.

From all nematode measures, omnivorous genera, the omnivorous trophic group, and maturity indices responded most consistently. Stronger pulses of agricultural practices (lower irrigation rates, higher fertilization levels, and bare soils) likely produced less stable soil environments, and thus supported less “mature” nematode communities (fewer omnivores and lower maturity indices). Although observed patterns for the above-mentioned nematode measures were relatively predictable, their absolute values were not. Significant differences in index values occurred temporally within experimental sites, and also across sites during similar sampling times.

Certain nematode families and genera were most closely associated with the magnitude of the maturity index. Rhabditidae and *Cephalobus* consistently were inversely related to maturity indices (*MI* and ΣMI), and the relationship was significant during half of the sampling events. *Acrobeloides*, *Wilsonema*, and *Aphelenchus* were often negatively associated with both *MI* and ΣMI (approximately one third of the sampling time). Positive correlations (60%, $P \leq 0.05$) with maturity indices were always observed for *Aporcelaimellus*, *Eudorylaimus*, Dorylaimidae, and miscellaneous plant parasites (*Meloidogyne*, *Xiphinema*, and *Pratylenchus*). *Prismatolaimus*, Chromadorida, and predatory mononchids and dorylaimids were usually positively correlated with maturity indices, but only 14-36% of sampling events were statistically significant. At the level of trophic groups, bacterivores and fungivores nearly always reduced maturity indices values. In contrast, plant parasites and omnivores almost always exhibited a significant positive effect on maturity indices. Although predators and algivores showed positive trends with maturity indices, their significance was marginal.

Nematode communities were related to several soil components. Fungal microflora, responding positively to nutrient enrichment, showed density-dependent patterns with fungal-feeding nematodes in the N-fertilizer rate experiment. Many nematode community measures were correlated with several macro- and micronutrients as well as with some aspects of sustainability (water use efficiency) in the irrigation experiment. No relationship between the majority of nematode indices and profitability was found, indicating that this characteristic of sustainability had little biological basis in our system.

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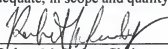
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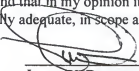
BIOGRAPHICAL SCKETCH

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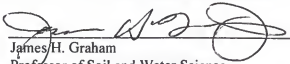
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Robert McSorley, Chair
Professor of Entomology and Nematology

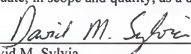
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Larry W. Duncan, Cochair
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


James H. Graham
Professor of Soil and Water Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


David M. Sylvia
Professor of Soil and Water Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1998


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